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Scientific Edition

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

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Volume XXX *JULY, 1941* Number 7
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A Simple Statistical Method for the Calculation of Mortality Percentages in Digitalis Assays*

By Harold N. Wright†

The percentage mortalities found by actual experiment in all procedures involving biological material are likely to show discordant results because of the variability in resistance of individuals, unless very large groups of animals are employed at each dosage. The latter is often not easily practicable, particularly where a relatively wide dosage range must be covered in the assay.

In digitalis assays by the official one-hour frog method, for example, it is usually most satisfactory to employ a relatively large number of small groups of frogs at different dosages, supplementing this procedure by the use of appreciably larger groups at the dosages in the critical range. Even so, discordant results frequently appear and the determination of clear-cut percentage mortality comparisons by purely experimental means without the use of an inordinately large number of animals is often difficult.

The calculation of the comparative strengths of (*a*) the standard tincture prepared from the U. S. Reference Standard Powder and (*b*) the preparation being assayed, according to the requirements of the United States Pharmacopœia (1), requires the injection of the two series of frogs to proceed "until the amounts of the percolate of digitalis and the standard preparation of digitalis, respectively, required to give equivalent percentages of positive reactions in approximately equal groups of frogs, are determined." The Pharmacopœia also states in the same paragraph that the injections must be repeated "if the largest doses of the respective preparations are not enough to give systolic standstill in at least 25 per cent of their respective groups or if more than 75 per cent of either group receiving the smallest doses are in systolic standstill." It would appear, therefore, that the "equivalent percentages of positive reactions" employed to "calculate the relationship of the activity of the preparation being assayed to the reference preparation" may lie anywhere within the range of 25 to 75 per cent mortality.

* Presented at the Group Meeting on Statistical Methods as Applied to Biological Assays of the Committee of Revision of the United States Pharmacopœia, Washington, D. C., May 13, 1940. A part of the funds used in this research was supplied by a grant from the Medical Research Fund of the University of Minnesota.

† From the Department of Pharmacology, University of Minnesota Medical School, Minneapolis, Minnesota.

Statistical methods of treatment of bioassay are not new. Trevan (2) showed that a sigmoid curve is obtained when the percentage of frogs showing a positive response to digitalis is plotted against the dosage. Later workers, notably Gaddum (3), Bliss (4) and Miller, Bliss and Braun (5) have demonstrated that this curve can be transformed into a straight line by plotting the logarithm of the dose against "an expression of the percentage effect in units derived from the normal frequency curve," termed "probits" by Bliss. The complexities of the theoretical arguments justifying such transformations deter many workers from employing these methods.

The method here presented is not intended to supplant these more elaborate methods, but simply to draw attention to a simple statistical method of increasing the accuracy of conclusions drawn from the experimental data without transformation of the data into artificial units which may tend to obscure the interpretation of the experimental results and prevent their comparison with the results of other workers.

The simple statistical method here presented for the more accurate calculation of percentage mortality statistics in digitalis assays than is given by the actual experimental data appears to have been first stated by Dragstedt and Lang (6). The method employs two assumptions which are logically quite sound and generally acceptable. These assumptions are: (1) that an animal which dies from a given dose would, under the same conditions, have died had it received any higher dose, and (2) that an animal which survives a given dose would also have survived had it received a smaller dose.

These assumptions are applied statistically by (1) integrating all survivals at higher dosages with those at the dosage under consideration and (2) simultaneously integrating all deaths at lower dosages with those at the dosage under consideration. Dragstedt and Lang clearly enunciated these assumptions and applied them in the above manner to the calculation of percentage mortality statistics of the effects of various respiratory stimulants in acute cocaine poisoning in rabbits.

Behrens (7) in 1929, apparently unaware of Dragstedt's publication, stated and applied the same fundamental assumptions to the calculation of a percentage mortality curve for the intravenous infusion of a strophanthin solution to frogs. He found that when his experimental determinations of the individual intravenous fatal dose on 140 frogs were analyzed as grouped data on the basis of the above assumptions, the percentage mortality curve agreed quite closely with the sigmoid dose-mortality curve previously determined experimentally on the same species of frogs (*Rana temporaria*) by Trevan.

Apparently unaware of the publications of either of these previous workers, Reed (8) and Reed and Muench (9) have more recently applied this method of calculating LD50 to problems of percentage mortality in irradiation and the biological titration of sera and viruses. Holck (10) has also recently discussed the applicability of the method to laboratory bioassay results.

The usefulness of the "Dragstedt method" of calculating percentage mortalities lies in the fact that the statistical procedure of simultaneous integration of survivals and deaths gives an accuracy to the percentage mortalities equivalent to that which would be obtained without integration only by the use of several times the number of animals employed with integration.

APPLICATION OF THE METHOD

The application of the Dragstedt double integration method to digitalis assays is shown in the following figures and tables. Table I shows the actual experimental data and the double integrated data for a single assay of the standard tincture prepared from the U. S. Reference Digitalis Powder. Forty-eight frogs were employed at nine dosage levels between 200 and 400 mg./Kg. In this particular assay (chosen to illustrate the utility of the statistical method rather than the perfection of the experimental results) the experiment shows a 50 per cent mortality at four dosage levels, namely 200, 250, 300 and 325 mg./Kg. Determination of mortalities from the integrated totals, however, smooths out these irregularities and places the LD50 at 322 mg./Kg. The graphic representation of these results is shown in Fig. 1. The effect of integrating survivals only, and of deaths only, is also shown in this

Table I.—Experimental and Integrated Mortality Statistics for a Single Assay Employing 48 Frogs of a Standard Tincture Prepared from the U. S. Reference Digitalis Powder by the U. S. P. One-Hour Frog Method (See Also Fig. 1)

Dose mg./Kg.	Experimental Data Alive	Dead	% Mort.	Integrated Data Alive	Dead	% Mort.
200	1	1	50	21	1	4.5
225	2	0	0	20	1	4.8
250	1	1	50	18	2	10
275	3	1	25	17	3	15
300	4	4	50	14	7	33.3
325	4	4	50	10	11	52.3
350	5	7	58	6	18	75
375	1	3	75	1	21	95.5
400	0	6	100	0	27	100

figure, from which it is apparent that this practice, which has been widely followed in bioassay statistical methods since its employment by Behrens, especially that of integrating survivals only, is not nearly as satisfactory as the Dragstedt double integration method. In the experiment here discussed use of survivals only has the effect of moving the LD50 too far to the right, thereby producing a fictitious lowering of the apparent toxicity of the drug, while use of deaths only assigns too great a toxicity to the drug.

The application of the method to the evaluation of the potency of an unknown tincture is illustrated in Fig. 2 and Table II. Figure 2 shows the experimental and double integrated curves for the simultaneous assays of the standard tincture prepared from the U. S. P. Reference Standard powder and the unknown tincture. The figures given in Table II have been read from this graph (plotted on coordinate paper). Since the U. S. P. one-hour frog method permits the calculation of corresponding mortality percentages throughout the 25–75 per cent range the comparative ratio strength of the unknown tincture to the reference standard tincture has been calculated for both curves at mortalities of 25, 40, 50, 60 and 75 per cent (Table II).

Table II.—Potency of Unknown Tincture of Digitalis in Terms of U. S. Reference Standard at Various Percentage Mortalities (See Also Fig. 2)

% Mortality	U. S. Ref., mg./Kg.	Unknown, mg./Kg.	Ratio Strength
	Experimental Curves		
25	225.0	275.0	75.0; 81.8
	265.5	300.0	88.3; 96.4
	312.0		104.0; 113.4
40	240.0	317.5	75.6
	255.0		80.3
	315.0		99.2
50	250.0	325.0	76.9
	322.5		97.0
60	327.5	350.0	93.6
75	350.0	361.0	97.0

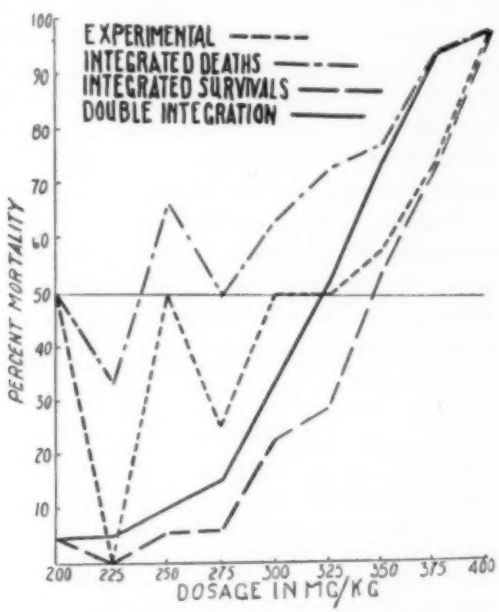

Fig. 1.—Graphic Representation of Various Methods of Integrating Survivals and Deaths in Digitalis Assays in Comparison with the Actual Experimental Data.

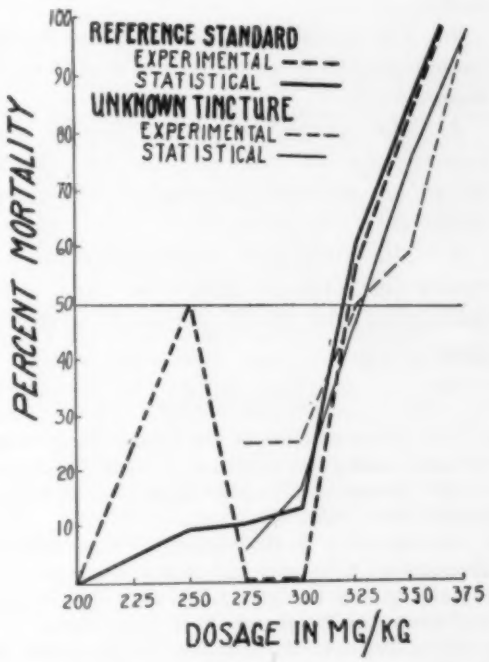

Fig. 2.—Experimental and Double Integrated Curves for Assay of U. S. Reference Standard Digitalis Tincture and Unknown Tincture.

Table II.—(*Continued*)

Dragstedt Curves

25	307.5	306.0	100.5
40	315.0	319.0	98.8
50	320.0	327.0	97.9
60	325.0	337.0	96.4
75	337.5	344.0	98.1

It will be noted that in the experimental curves a mortality of 25 per cent is shown at three dosage levels for the U. S. P. Reference Standard tincture and at two dosage levels for the unknown tincture, thus giving six possible ratio strengths for the unknown tincture at this percentage mortality, ranging from 75.0 to 113.4 per cent of the U. S. P. potency. The application of the double integration procedure to the two experimental curves, however, completely smooths out these irregularities and gives curves for the corresponding mortality percentages of the two preparations to be composed, which run almost parallel throughout the 25–75 per cent mortality range.

Taking the figures from the Dragstedt curves the potency of the unknown tincture varies through the corresponding percentage mortality values only between 96.4 and 100.5 per cent of U. S. P. potency, with a mean value of 98.3 per cent and a probable error of the mean ±0.403 per cent.

SUMMARY

1. A simple statistical method is presented for the rapid and accurate calculation of comparative potencies in digitalis assays.

2. The method employs the simultaneous integration of survivals and deaths at all dosage levels.

3. The method does not require any transposition of units and the results obtained are directly comparable with those obtained by other workers.

4. The integrated results have an accuracy of approximately ±1 per cent throughout the 25–75 per cent mortality range.

REFERENCES

(1) Pharmacopœia of the United States, 11th Edition, Mack Printing Co., Easton, 1936.

(2) Trevan, I. W., *Proc. Roy. Soc.* (London), Ser. B., 101 (1927), 483.

(3) Gaddum, J. H., *Med. Res. Council (Brit.), Special Rept. Series No. 183*, 1933.

(4) Bliss, C. I., *Science*, 79 (1934), 409; *Quart. J. Pharm. and Pharmacol.*, 11 (1938), 192.

(5) Miller, L. C., Bliss, C. I., and Braun, H. A., JOUR. A. PH. A., 28 (1939), 644.

(6) Dragstedt, C. A., and Lang, V. F., *J. Pharmacol.*, 32 (Dec. 1927), 215.

(7) Behrens, B., *Arch. Exp. Path. Pharmakol.*, 140 (1929), 237.

(8) Reed, L. J., "Statistical Treatment of Biological Problems in Irradiation," Chap. 2, in Duggar, B. J., "Biological Effects of Radiation," McGraw-Hill, New York, 1936.

(9) Reed, L. J., and Muench, H., *Am. J. Hyg.*, 27 (May 1938), 493.

(10) Holck, H. G. O., *Am. J. Pharmaceut. Ed.*, 4 (1940), 602.

A Pharmacological Study of Some New Synthetic Hypnotics*

By John W. Nelson, Stanley C. Lyster and George F. Cartland

The studies here reported were undertaken with the purpose of evaluating in a preliminary way, the hypnotic activity of a large group of synthetic compounds, most of which have not previously been described in the literature. All of the compounds here studied, with the exceptions noted in Table I, were prepared by Blicke and Centolella (1, 2). The rat has generally been regarded as the most suitable animal for such studies, and the intraperitoneal route has been adopted by us as the most sensitive method of estimating hypnotic activity in a group comprising compounds of widely different solubilities. Fitch and Tatum (3) have suggested the intraperitoneal route as closely approximating slow intravenous injection. For references to the literature and a discussion of methods we refer to this paper.

Further studies on the more effective members of the series, involving measurement of induction time, and duration of sleep by oral administration in rabbits and intraperitoneal injection in rats are included in this report.

EXPERIMENTAL

Rat Experiments.—Healthy young adult male Wistar strain albino rats raised in our animal colony and weighing 175 to 300 Gm. were used in all experiments included in this report. No animal received more than one injection in order to avoid any tolerance for the material injected. The rats were not starved previous to injection since preliminary ex-

* From the Research Laboratories, The Upjohn Company, Kalamazoo.

Table I.—Hypnotic Activity by Intraperitoneal Injection in Rats

	Substituted Acetylureas	Therapeutic Index M. L. D./M. H. D. =
1.	*N*-Methyl-diethyl	700/300 = 2.3
2.	Ethyl-propyl	>750/>750 = ...
3.	*N*-Methyl-ethyl-butyl	500/300 = 1.7
4.	Ethyl-butyl-α-bromo	>740/>740 = ...
5.	Ethyl-amyl	>1000/>1000 = ...
6.	Ethyl-hexyl	>500/>500 = ...
7.	Ethyl-β-cyclohexylethyl	>1000/>1000 = ...
8.	Di-β-cyclohexylethyl	>950/>950 = ...
9.	β-Phenylethyl	>500/>500 = ...
10.	Ethyl-benzyl	>600/>600 = ...
11.	Ethyl-β-phenylethyl	>1000/>1000 = ...
12.	Di-β-phenylethyl	>500/>500 = ...
13.	Propyl-β-phenylethyl	>500/>500 = ...
14.	Isopropyl-β-phenylethyl	>600/>600 = ...
15.	Allyl-β-phenylethyl	>750/500 = >1.5
16.	Butyl-β-phenylethyl	>500/>500 = ...
17.	Isobutyl-β-phenylethyl	>600/>600 = ...
18.	β-Cyclohexylethyl-β′-phenylethyl	>500/>500 = ...
19.	Ethyl-γ-phenylpropyl	>750/>750 = ...
20.	Ethyl-δ-phenylbutyl	1000/1000 = 1.0
21.	Ethyl-ε-phenylamyl	>500/>500 = ...
22.	Ethyl-ζ-phenylhexyl	>1000/>1000 = ...
23.	Ethyl-cinnamyl	>500/>500 = ...
24.	Ethyl-methyl	>500/350 = >1.4
25.	Diethyl	500/250 = 2.0
26.	Ethylbutyl	>500/400 = >1.25
27.	α-Bromodiethyl	380/130 = 2.9
	Substituted Acetamides	
28.	Diethylthio	100/80 = 1.25
29.	*N*-Methyldiethyl	>600/500 = >1.2
30.	*N*-Butyldiethyl	>600/500 = >1.2*
31.	Ethylbutyl	350/100 = 3.2
32.	*N*-Methylethylbutyl	300/150 = 2.0*
33.	*N*-Ethylethylbutyl	250/200 = 1.25*
34.	*N*-β-Hydroxyethylethylbutyl	500/300 = 1.6
35.	*N*-Butylethylbutyl	300/... = ...*
36.	Ethylamyl	400/100 = 4.0
37.	Ethylhexyl	>450/150 = >3.0
38.	Ethyl-β-cyclohexylethyl	>500/>500 = ...
39.	*N*-Methylethyl-β-cyclohexylethyl	>500/>500 = ...
40.	*N*-Ethylethyl-β-cyclohexylethyl	>500/>500 = ...
41.	*N*-β-Hydroxyethylethyl-β′-cyclohexylethyl	>500/200 = >2.5
42.	*N*-Butylethyl-β-cyclohexylethyl	>1000/>1000 = ...
43.	Di-β-cyclohexylethyl	>650/>650 = ...
44.	*N*-Methyldi-β-cyclohexylethyl	>750/>750 = ...
45.	*N*-Ethyldi-β-cyclohexylethyl	>750/>750 = ...
46.	Butyl-β-cyclohexylethyl	.../... = ...
47.	*N*-Methylbutyl-β-cyclohexylethyl	>800/>800 = ...
48.	*N*-Ethylbutyl-β-cyclohexylethyl	>500/>500 = ...
49.	*N*-β-Hydroxyethylbutyl-β′-cyclohexylethyl	>500/>500 = ...
50.	*N*-(Diethylacetyl)-morpholine	600/400 = 1.5
51.	*N*-(Ethylbutylacetyl)-morpholine	450/175 = 2.6
52.	*N,N′*-bis-(Diethylacetyl)-ethylenediamine	>2000/>2000 = ...
53.	Ethylbenzyl	425/175 = 2.4
54.	Ethyl-β-phenylethyl	325/120 = 2.7
55.	*N*-Methylethyl-β-phenylethyl	400/200 = 2.0*
56.	*N*-Ethylethyl-β-phenylethyl	200/150 = 1.3*
57.	Di-β-phenylethyl	>500/>500 = ...
58.	*N*-Methyldi-β-phenylethyl	>500/>500 = ...
59.	*N*-Butyldi-β-phenylethyl	>500/>500 = ...
60.	β-Cyclohexylethyl-β′-phenylethyl	>300/>300 = ...
61.	Propyl-β-phenylethyl	500/200 = 2.5
62.	*N*-Methylpropyl-β-phenylethyl	>200 <500/200 = ...*
63.	*N*-Ethylpropyl-β-phenylethyl	400/200 = 2.0*
64.	*N*-β-Hydroxyethylpropyl-β′-phenylethyl	400/200 = 2.0
65.	*N*-Butylpropyl-β-phenylethyl	>500/>500 = ...*
66.	Isopropyl-β-phenylethyl	400/150 = 2.6
67.	*N*-β-Hydroxyethylisopropyl-β′-phenylethyl	450/250 = 1.8*
68.	Allyl-β-phenylethyl	350/125 = 2.8
69.	Butyl-β-phenylethyl	>1000/>1000 = ...
70.	*N*-Methylbutyl-β-phenylethyl	>1000/500 = >2.0*
71.	*N*-Ethylbutyl-β-phenylethyl	600/600 = 1.0*
72.	*N*-β-Hydroxyethylbutyl-β′-phenylethyl	400/200 = 2.0*
73.	*N*-Butylbutyl-β-phenylethyl	>1000/>1000 = ...
74.	Isobutyl-β-phenylethyl	450/125 = 3.6
75.	Ethyl-γ-phenylpropyl	>500/400 = >1.25
76.	Ethyl-δ-phenylbutyl	600/250 = 2.4
77.	Ethyl-ε-phenylamyl	>750/150 = >5.0
78.	Ethyl-ζ-phenylhexyl	>1000/>1000 = ...
79.	Ethylcinnamyl	400/200 = 2.0
80.	Ethylmethyl	>1000/>1000 = ...
81.	Diethyl	>500 <1000/>300 = ...
82.	Ethylpropyl	500/200 = 2.5
83.	Cyclohexyl	600/600 = 1.0
84.	β-Phenylethyl	>500/300 = >1.7
85.	*N*-α-Hydroxymethyldiethyl	>1000/>800 = ...
86.	Tetramethylsuccinimide†	15/... = ...*
87.	Ethyl isoamyl	425/110 = 3.8

Compounds 1 to 23, inclusive, described in Table II (Reference 1).
Compounds 28 to 51, inclusive, described in Table I (Reference 2).
Compounds 53 to 79, inclusive, described in Table II (Reference 2).
Compounds 24 to 26, inclusive, and 80 to 86 inclusive prepared by Blicke and Centolella but not published.
Compound 27 (carbromal U. S. P.) was used as reference standard.
Compound 87 prepared by Dr. H. G. Kolloff.
* Convulsant action.
† Compound 86 is not a substituted acetamide.

periments showed that only a 5 per cent decrease in body weight resulted after 16 hours' fasting.

All compounds were suspended in 5 per cent acacia solution immediately before injection in order to avoid any decomposition of the compounds. Recourse to solvents other than 5 per cent acacia solution is precluded for the reason that they would be toxic in the volume range required by the tests. The material was injected intraperitoneally as a 5 per cent suspension into rats and the time of injection, onset and duration of sleep were recorded. The rats were observed closely for sleep during the first 3 hours after injection and any dose which failed to produce sleep during this period was considered sub-hypnotic. All animals were observed for delayed deaths for a period of seven days after injection.

Table II.—Hypnotic Activity by Oral Administration in Rabbits

	Compound	M. L. D.	M. H. D.	M. L. D. / M. H. D.	Induction Time at Approx. 60% M. L. D.	Duration of Sleep at Approx 60% M. L. D.
36.	Ethyl-*n*-amyl acetamide	1750	400	4.4	20 min.	>5 hours
54.	Ethyl-β-phenylethyl acetamide	>2000	750	>2.6	35 min.	>4 hours
31.	Ethyl-*n*-butyl acetamide	1250	150	8.3	17 min.	3 hours
87.	Ethyl-isoamyl acetamide	>2000	300	>7.0	.....	.....
68.	Allyl-β-phenylethyl acetamide	>2000	>2000	...	.....	.....
53.	Ethyl-benzyl acetamide	>3000	1000–1500	>2.0	.....	.....
37.	α-Bromodiethyl acetylurea (carbromal U. S. P. XI)	1500	175	8.6	Approx. 1 hr.	>6 hours

The minimum hypnotic dose, or M. H. D., is defined as the minimum dosage at which at least 50 per cent of the injected animals are put to sleep. "Sleep" is defined as the period during which the animals do not assume a normal position when placed on their backs, and during which the animals respond to pinching the tip of the tail by only unsuccessful attempts at righting themselves. The minimum lethal dose, or M. L. D., is defined as the smallest dose killing at least 50 per cent of the injected animals. The therapeutic index is the ratio of minimum lethal dose to minimum hypnotic dose or M. L. D./M. H. D.

Compounds which failed to produce sleep at a dose of 500 mg. per Kg. were considered as relatively inactive and their therapeutic ratios were not determined. The more effective hypnotics were evaluated using 20 to 40 rats on each compound. Thus, it is possible to select from the entire group of 87 compounds, six which were most effective for producing hypnosis in rats. These compounds were subjected to more detailed study in rats and also orally in rabbits. Carbromal (No. 27) was injected in parallel as a reference standard.

Rabbit Experiments—Mature healthy rabbits weighing 1500 to 3000 Gm. were used for testing the effectiveness of the six more active compounds by oral administration. The material was suspended in 5 per cent acacia solution and administered by stomach tube to rabbits starved for 12 hours. The tube was rinsed with about 5 cc. of 5 per cent acacia solution to insure that the full dose was administered. The M. H. D., M. L. D. and therapeutic ratio is defined as in the rat experiments except that sleep in the rabbit is defined as the period during which the animals do not assume a normal position when placed on their backs, and during which the animals do not right themselves when pinched in the thigh muscles with thumb and forefinger.

Results.—The pharmacologic data based on the results obtained from 1053 rats and 97 rabbits are recorded in Tables I and II. Of the compounds studied, the acid amides are outstanding as a group in that all of the hypnotics having a satisfactory M. H. D. and therapeutic ratio are included in this group. As a group, the acetyl ureas are relatively inefficient hypnotics for the rat, which may in part be due to their poor solubility. It is interesting to observe that, with the exception of number 86, all of the compounds showing convulsant action are *N*-substituted acid amides. This convulsant action is apparently reduced by the substitution of an ^-OH in the alkyl group attached to *N*- or by β-cyclohexylethyl in the α-position.

Of the six compounds studied orally in rabbits, only one, ethyl-*n*-butyl acetamide, appears to be more active than carbromal. The other five are much more effective by intraperitoneal injection in rats than by oral administration in rabbits. This discrepancy may be due to poor absorption from the stomach or possibly to destruction in the gut.

SUMMARY

Eighty-seven compounds have been studied by intraperitoneal injection in rats for estimation of the therapeutic ratio Minimum Lethal Dose/Minimum Hypnotic Dose. Six of the most promising compounds of this group have been studied further by oral administration in rabbits. Only one compound, ethyl-*n*-butyl acetamide, was found to be more active than carbromal by both tests.

REFERENCES

(1) Blicke, F. F., and Centolella, A. P., *J. Am. Chem. Soc.*, 60 (1938), 2923.

(2) Blicke, F. F., and Centolella, A. P., *Ibid.*, 60 (1938), 2924.

(3) Fitch, R. H., and Tatum, A. L., *J. Pharmacol*, 44 (1932), 325.

"If you, or I, can ultimately give to the world a single volume, or line, or thought which will enable our fellow men and women to enjoy life a little more fully, or endure it a little more easily, then we shall not altogether have laboured in vain."—Ian Hay

Pressor Drugs. I

Chemistry and Pharmacology of an Analogue of Epinephrine*

By James C. Munch,† Vincent H. Gattone and Harry J. Pratt

The increasing number of clinical reports of the effectiveness of pressor drugs in the relief of certain conditions in the respiratory tract, chiefly asthma and hay fever, led to this coöperative investigation.

The original injection therapy has been supplemented by inhalational therapy with increased benefit to the patients (3, 5, 9). The chemistry of the drugs which increase the blood pressure results from the pioneer investigations of Abel, whose first report was published in 1889 (1) and which has been supplemented by chemical and pharmacological studies by a number of workers throughout the world, notably Aldrich, Barger, Dale, Elliott, von Fürth, Langley, Schäfer and Oliver, and Takamine, whose results have been reviewed by Schultz (10), May (6) and Hartung (4). A detailed discussion is also presented by Cushny (2), and by Munch (7), from the standpoint of isomeric substances. The most widely used product of this group is epinephrine which is officially recognized by U. S. P. XI (8).

In this paper of the series, information is presented on the chemical and pharmacological properties of a synthetic product, racemic methylamino-hydroxy-ethyl-dihydroxy-benzene, and the hydrochloride of this base, the solution of which is known as "Vaponefrin." Comparable studies have been made on U. S. P. Reference Standard Epinephrine and on some of the commercial epinephrine solutions.

EXPERIMENTAL

In these investigations synthetic material was furnished by the Vaponefrin Company from four different lots of the product. These were uniform in behavior. The base is a fine white powder; the hydrochloride somewhat resembles epinephrine hydrochloride, except that it is snowy white. In general, the solubilities of the two products correspond. The method of production of vaponefrin will be considered in a subsequent paper of this series.

A large number of chemical studies have been made in an effort to determine the reactions of vaponefrin and epinephrine, and to develop methods of differentiation. In a number of chemical tests the colors produced by both substances were so nearly identical that the reaction did not appear suitable for this purpose. Nine tests were found in which different types of response were so obvious that they appeared suitable for chemical distinction, qualitatively. The quantitative applications of these color reactions to the pure products and to mixtures of vaponefrin and epinephrine are being studied further.

The different types of responses obtained with U. S. P. Reference Standard Epinephrine and with vaponefrin are given in Table I. The difference in color following the application of concentrated sodium hydroxide to the dry base serves to dis-

Table I.—Chemical Differentiation between U. S. P. Epinephrine and Vaponefrin

Tests Applied	U. S. P. Epinephrine	Vaponefrin
	Powder	
1. Optical rotation	−50° to −53.5°	NO READING
2. Dry base treated with conc. NaOH cold solution	REDDISH BROWN gradually deepening in color	Amber—GREENISH BROWN—brown
	Solution 1: 1000	
3. Oxidation (by passing air through solution)	Pink—VIOLET—brown	Pink—AMBER—brown
4. Iodine T.S.	Pink—pinkish brown to RED upon standing over night	Pink—pinkish brown to GREEN upon standing over night
5. Krull method	CLEAR amber to cherry-red upon standing	MILKY WHITE PPT. CLEARING UP ON SHAKING to amber to cherry-red upon standing
6. Sodium hydroxide solution	Amber—cherry-red upon standing	Amber to BROWN upon standing, after 1 to 2 days to cherry-red
7. Phosphomolybdic acid T.S.	VERY PALE amber	DARK amber or yellowish brown
8. Ferric chloride T.S.	EMERALD-green—cherry-red—brown upon standing	BLUISH green—cherry-red—brown upon standing
9. Potassium ferricyanide T.S.	Red color upon standing HALF hour	Red color upon standing TWO to THREE hours

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tinguish between these products; epinephrine giving a reddish brown color and vaponefrin a greenish brown color. The characteristics in which differences are noted are indicated in capital letters in Table I. In tests on the solutions the iodine test No. 4 and the ferric chloride test No. 8 were the most suitable for differentiation.

A colorless solution was prepared for the pharmacological investigations containing two Gm. of the base in sufficient distilled water to furnish 100 cc. of solution. Before bringing up to final volume, enough $N/10$ HCl was added to complete solution and to produce a p_H of 4.0. This solution was packaged in half-ounce, amber glass bottles. For deterioration studies, solutions were prepared as needed. For other pharmacological investigations 0.5 per cent chloretone was used as a preservative, and some of this material was included in the deterioration studies as a control on the solution without chloretone. Both types of vaponefrin solutions were also used in the inhalational studies.

Toxicity tests were made on vaponefrin and U. S. P. Epinephrine. The results obtained following oral administration to mice, and following intraperitoneal injections to mice and rats are consolidated in Table II. The results presented are stated in terms of mg. of the base per Kg. of body weight. It may be noted that epinephrine is about one and a half times as toxic as vaponefrin base, slightly different ratios being obtained in the different types of tests.

Table II.—Acute Toxicity to Animals

Animal	Method of Administration	Epinephrine, mg./Kg. Survived	Epinephrine, mg./Kg. Killed	Vaponefrin, mg./Kg. Survived	Vaponefrin, mg./Kg. Killed
Mice	Oral	50	60	60	80
Mice	Intraperitoneal	2.5	5	5	10
Rats	Intraperitoneal	0.75	1.0	1.0	1.5

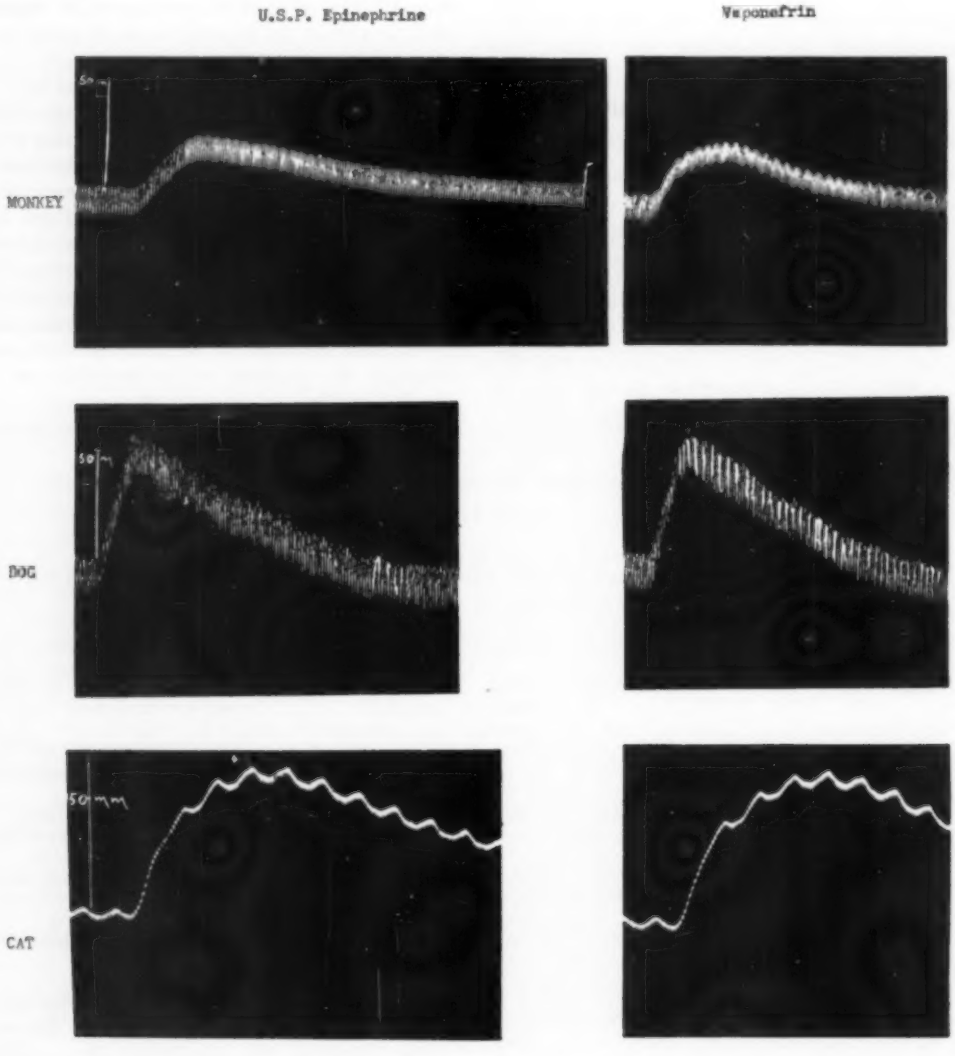

Fig. 1.—Blood Pressure Tracings.

To supplement these types of administration, a series of rabbits were obtained from the same source of supply for inhalational studies. A series of vaponefrin vaporizers were obtained for administration of the vapor, and the orifices reduced by sealing Pyrex tubing to the outlets so that they might be used on rabbits. In groups of animals, 1, 5 or 10 inhalations were given twice daily directly into the mouth as the rabbits inhaled so that the vapor might be carried into the lungs. Animals were treated five days weekly; these studies were started in September, 1939, and will be continued for at least a year. In the period of eight months, a few animals have died from extraneous causes and some have been sacrificed for histopathological study. The other animals of the group are growing at a normal rate and appear to be in healthy condition. Some of these rabbits have received about four thousand inhalations.

As further controls on the inhalational study, additional rabbits from the same source have been given 5 cc. of a 1:200 dilution of vaponefrin solution daily for eight months. A total of about 90 mg. of vaponefrin has been given over a period of eight months without producing any changes in growth or appearance.

A number of studies of the pressor action have been made. Figure 1 shows portions of responses observed following intravenous injections of epinephrine and of vaponefrin to anesthetized cats, dogs and monkeys (*Macacus rhesus*). It will be observed that the rate of increase in blood pressure is similar after the administration of either product to the same species of animal. In general, more rapid elevations were observed in dogs. The pressor potency of vaponefrin base was found to be about two-thirds the pressor potency of epinephrine base. By properly modifying the concentrations of solutions prepared, the same intensities of pressor response were readily obtained.

In Chart 1 the epinephrine and vaponefrin doses intravenously injected into monkeys were 8 and 20 gamma; into dogs 7 and 12 gamma and into cats 10 and 16 gamma, respectively. These tracings are presented to show the qualitative similarity of response, rather than the quantitative pressor values.

Deterioration studies were undertaken on a series of samples of epinephrine and on a lot of vaponefrin (No. 81,839) with a p_H of 4.0. One-half ounce, amber bottles of epinephrine and of vaponefrin under similar conditions were placed in: (1) an incubator at 55° C.; (2) an incubator at 37° C.; (3) at room temperature in the laboratory; and (4) in a refrigerator maintained at a temperature of about 5° C. Samples were removed from time to time for physical examination, p_H determination and measurements of pressor potency by the U. S. P. XI Epinephrine assay on dogs. These samples were prepared and stored on October 12, 1939.

No changes have been observed in the vaponefrin solutions stored under conditions 1, 2 or 4, over a period of five months. A portion of the bottles stored at room temperature (in the dark) showed no change in color or potency. Another portion, which had been stored in direct sunlight but unopened until taken for test, showed no changes in the color or potency. However, a third portion, stored in sunlight and opened daily, showed progressive darkening color to muddy brown after two months, with the appearance of a slight precipitate, in the bottles containing vaponefrin. The contents of these bottles showed a progressive lowering of p_H. No significant changes in physiological activity were detected when the contents of these bottles were tested against freshly prepared vaponefrin solution of the same concentration.

The solutions of epinephrine in bottles, stored in the sunlight and opened daily, showed prompt color changes, reaching a maximum darkening within about two weeks. There was a progressive decrease in pressor potency associated with the development of the darker color. About half of the pressor activity was lost during a period of two to four weeks. The comparative deterioration studies of epinephrine and vaponefrin will be reported in a subsequent paper.

CONCLUSIONS

1. Methods of chemical differentiation between U. S. P. Epinephrine and vaponefrin (racemic methylamino-hydroxy-ethyl-dihydroxy-benzene hydrochloride) have been developed and the qualitative differences presented.

2. In acute toxicity tests on animals, vaponefrin was about two-thirds as toxic as epinephrine, following injections.

3. In chronic inhalational studies on rabbits, no toxic effects have been observed following inhalation of quantities up to four thousand inhalations of vaponefrin.

4. The pressor responses of cats, dogs and monkeys to epinephrine and to vaponefrin were similar. Epinephrine is 50 per cent stronger in pressor potency.

5. Vaponefrin solutions were more stable than epinephrine solutions when stored under similar conditions. Discolored vaponefrin solutions retained their pressor potency.

REFERENCES

(1) Abel, J. J., *Z. physiol. Chem.*, 28 (1889), 318.
(2) Cushny, A. R., "Biological Relations of Optically Isomeric Substances" (1926).
(3) Eckman, M., and Barach, A. L., *Modern Hospital*, 52 (1939), No. 2.

(4) Hartung, W. H., "Chemical Reviews," 9 (1931), 389.
(5) James, P., "Therapeutics of the Respiratory Passages" (1884).
(6) May, P., "The Chemistry of Synthetic Drugs" (1939).
(7) Munch, J. C., "Bioassays" (1931).
(8) The Pharmacopœia of the United States, 11th Edition (1936).
(9) Richards, D. W., Barach, A. L., and Cromwell, H. A., *Am. J. Med. Sci.*, 199 (1940), 225.
(10) Schultz, W. H., *Hyg. Lab. Bull.*, No. 55 (1909).

The Irritant Effect of Aloin. Preliminary Note

*By Melvin W. Green**

America produced, in 1931, $20,461,000 worth of laxatives (1). But in spite of the economic value of the cathartic drugs, little serious attention has been given to the chemistry, pharmacology or physiology of laxation.

For some time the author has been interested in tools for studying these problems and applying them to the anthraquinone-bearing drugs such as senna, aloes, cascara and rhubarb.

The most obvious study, to begin such a project, is the assay of these drugs for cathartic value. We have already worked out some methods for assaying which, while not sharply quantitative, are very useful as tools (2) (3).

But in addition to assay methods, it is important to find measures of untoward effects which may or may not contribute to catharsis. Alvarez (4) has shown that if rabbits are purged and then killed by a blow on the head, isolated gut strips from such animals are considerably atonic. In addition, the animals appeared to be depressed. Larson and Bargan (5) have also shown in surgical dogs that laxatives upset the normal gut movements so that, in the case of the more drastic purges, it is actually days before the movements become normal.

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EXPERIMENTAL

In order to compare some unofficial aloins with the U. S. P. Aloin, the following technique for determining the irritation of the mucosa was developed. Well-nourished, healthy guinea pigs, weighing around 300 Gm., were subjected to Nembutal anesthesia (30 mg. per Kg.). An incision was made in the abdomen and the ascending colon exposed. A 5-cm. loop of large intestine was tied off with thread and 0.5 cc. of a solution of the aloin in question injected into the loop, the loop returned to the abdomen and the abdomen sewed up loosely. The animal was kept warm, and after an hour the gut loop was again brought to the surface and the isolated loop cut away. The excised loop was carefully washed free of fecal matter, opened and the degree of redness of the interior surface compared with a similar loop from a control animal. The control animal received a solution of aloin, Merck, in a concentration of 1.0–1.5 mg. per cc. The concentration of test aloin and volume of solution injected were always the same as in the control.

Table I indicates the results obtained with some unofficial aloins. These aloins were prepared by the Purdue University School of Pharmacy and submitted to this laboratory for testing. The aloins were extracted from several different aloes and by new and different processes. The standard was considered to have an irritating effect of two plus and the other aloins compared with it. Based on practice determinations, a change of one plus in either direction is of doubtful significance. From these data the unofficial aloins are probably no more irritant than the official product.

Table I.—Effect of Various Aloins on Intestinal Mucosa

Aloin	Inflammation
Standard	++
Socratine aloin	+
Barbadoes aloin	+++
Cape aloin No. 1	++
Cape aloin No. 2	+ ?
Cape aloin No. 3	+

It was thought advisable to check this irritation with actual griping in human subjects. This phase of the problem is in the preliminary stages, but of the aloins tested, none of them is as griping, in 1/4 gr. doses, as is the commercial product.

SUMMARY

In order to compare some unofficial aloins with the U. S. P. Aloin, the author has developed a technique for determining the irritating effect on the intestinal mucosa.

The author wishes to acknowledge with gratitude the provision of the aloin specimens by Dr. Jordan of Purdue University and the generous gifts of guinea pigs supplied by the William S. Merrell Drug Company.

REFERENCES

(1) *Biennial Census of Manufactures*, U. S. Department of Commerce, Bureau of Census, Washington (1933), p. 337.

(2) Green, King and Beal, JOUR. A. PH. A., 25 (1936) 107.

(3) Green, King and Beal, *Ibid.*, 27 (1938) 95.

(4) Alvarez and Taylor, *J. Pharmacol.*, 10 (1917) 365.

(5) Larson and Bargan, *Arch. Surgery*, 27 (1933), 1120.

Preliminary Studies with Nepeta Glechoma*

J. Howard Graham and Louise M. Bitto

Nepeta Glechoma, Benth (*Glechoma hederacea*, L), more commonly called Ground Ivy or Gill-over-the-ground, is beautiful when grown under controlled conditions in a rock garden, but is a pesty weed when it escapes. The plant is a common member of the *Labiatæ*, naturalized from Europe and recognized by square stems and more or less two-lipped corollas.

One of the authors was led by curiosity to ascertain something of the chemical nature of this member of the mint family. Unfortunately, much of the "weed" had been discarded before the inspiration came to attempt an investigation. Upon consultation with the U. S. Department of Agriculture, there were furnished two brief references (1, 2), stating that Schimmel and Co. had distilled 0.03 per cent of the volatile unpleasant-smelling oil from the plant; the oil has a dark green color; that H. Haensel had obtained 0.064 per cent of oil from the dry herb, with a density of 0.9296 at 21° C.; that the oil showed traces of aldehyde or ketone; and that tannin and bitter substances were present. No other references have been found to this variety of Nepeta. However, brief statements were found concerning the following varieties of Nepeta: *N. Botryoides* (3); *N. Botryoides-Artemisi* S. minor (3); *N. Bucharia*, *N. Grandiflora* and *N. Podstachys* (4); *N. Cataria* (seeds) (5).

* Contribution from the Department of Organic Chemistry, Temple University, School of Pharmacy, Philadelphia, Penna.

EXPERIMENTAL

Preparation of the Material.—During the spring and summer of 1939 the creeping Nepeta was pulled from the ground at a time when its blue flowers were dropping. Flowers (Fig. 1) and fine roots were severed as completely as possible. The plant was washed frequently until no dirt settled from the wash water. The plant was drained and dried and a distillation was made with steam. More plant was gathered later with flowers and some seed (Fig. 2). From this crop a small vial of seed was obtained. During the summer other crops were obtained, washed thoroughly and air-dried for approximately six months. Evidently, during this long drying process some oil was lost.

Steam Distillation.—The cut or crumpled plant, largely the leaves with their oil sacs (Fig. 3), was steam-distilled with a collection of 1000 cc. to 2000 cc. of distillate. At first, small droplets of a faintly yellow-colored oil appeared, that later disappeared into the water of the distillate making it faintly milky. This was shaken with redistilled chloroform, the chloroform evaporated spontaneously and the resudue weighed to constant weight. The extracted water retained a faint odor of the oil, was slightly milky and the emulsion was not disturbed by any solvent, but it cleared immediately upon addition of dilute NaOH. Dilute HCl did not do this.

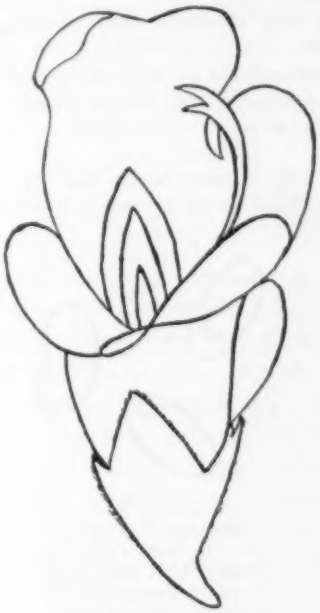

Fig. 1.—Flower.
Flower is bell-shaped, 1 cm. in length, violet-blue in color with a deep maroon-red center. Corolla is about thrice the length of the calyx. Calyx is covered with non-glandular trichomes. Anther cells diverge at right angles, each pair approximate and forming a cross.

Physical Properties of the Oil.—The residue from the chloroform was reddish yellow and it had an unpleasant odor. Chloroform, ether, benzene and alcohol were found to be good solvents. If a solution was filtered with a small amount of Darco, the reddish color disappeared, and the recovered oil substance dried to a waxy, but apparently crystalline residue, especially upon slight chilling. Probably the melting temperature is slightly below room temperature. Neither the melting temperature nor the refractive index of the material could be taken because of the very small amount of material on hand. When softened and drawn into a capillary tube, the specific gravity was found to be 1.000.

Physical properties of the Distillate.—Of the semi-crystalline, waxy substance, only 0.0730 Gm. was obtained from four distillations. This darkened upon exposure to the air, but could be purified by redistillation with steam. It showed probable phenolic and aldehydic characteristics, and unsaturation.

Seed.—An effort to obtain an ether extract of the crushed seeds met with failure because of an unpredictable accident.[1] The seed gave 7.9 per cent of ash.

Plant Ash.—A large burlap bag full of the plant had been dried for six months. It was reduced to fine powder with a meat grinder. Seven determinations of the ash contents gave an average of 11.63 per cent. Spectroscopically, the ash revealed K, Na, Ca, Ba and traces of Li. With methyl alcohol and sulfuric acid, the ash showed traces of boron. A sodium carbonate fusion showed Fe, Al, Si, Ca and Mg. Treated with HCl, the ash showed the presence of carbonates. A trace of Mn was shown by the borax bead test. Sodium fusion of the dried plant gave a positive test for N with traces of Cl, P and S. Carbon, hydrogen and oxygen, of course are present in the chlorophyll.

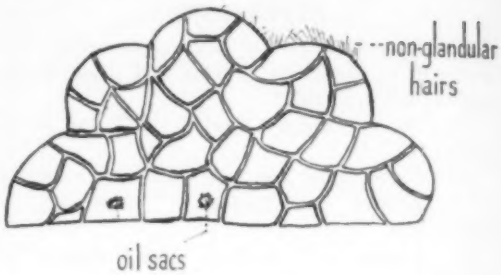

Fig. 3.—Leaf.

The leaf is 1 to 2 cm. in diameter, petioled, round, kidney-shaped, crenate and green on both sides; covered with non-glandular trichomes; venation reticulate. Oil sacs appear as depressions lighter in color than surrounding area.

Table I.—Results of Steam Distillation

Plant Condition	Weight of Material, Gm.	Volume of Distillate, Cc.	Waxy Product		pH of	
			Gm.	%	Distillate	Plant Liquor
Air-dried	100	2000	....	....	7.47	5.47
Fresh	109	1300	0.0162	0.0148	5.89	5.55
Very dry	100	1500	0.0233	0.0233	6.49	5.67
Very dry	100	2000	0.0177	0.0177	6.90	5.43

Fig. 2.—Seed.
The seed is about 0.5 mm. in diameter and about 1 to 2 mm. long. Ovoid in shape, slightly roughened exterior, seemingly pitted; vanilla-brown in color. White hilum at base is loop-shaped; seed coat tough.

[1] Plumbers opened all faucets, two floors below, and thereby drained the water condenser of an ether extraction Soxhlet apparatus, with resulting destruction of apparatus and contents.

The thanks of the authors are extended to Dr. Frank Eby for valuable assistance in his botanical laboratory.

REFERENCES

(1) Gildermeister and Hoffmann, "Die Aetherischen Ole," 3rd Edition, Vol. 3, page 689.

(2) Wehmer, "Die Pflanzenstoffe," 2nd Edition, Vol. 2, page 1032.

(3) Pigulevski, *et al.*, *Gen. Chem.* (U. S. S. R.) (1935), 1798–1800; through *Chem. Abstr.*, 30 (1935), 3940[4].

(4) Kudryashev, S., "Trav. Sta. Exp. Sci. Asie Cent. pour les huiles ess.," No. i, 1932; through *Chem. Abstr.*, 28 (1932), 5599[6].

(5) Peppino, Liotta, *Rev. Parf.*, 4 (1024), 10; through *Chem. Abstr.*, 18 (1924), 1731[6].

Isotonic, Buffered and Preserved Intranasal Ephedrine Sulfate Solutions

*By G. A. Tozer and Louis Arrigoni**

The use of nose drops and nasal sprays in the alleviation of the symptoms of head colds and other ailments producing congestion of the nasal passages has become exceedingly widespread in recent times. In spite of the popularity of this form of medication, very few of the nasal preparations obtainable at the present time are prepared with sufficient scientific forethought to produce the most effective product possible from the materials at hand. Many of the nose drops and sprays, still in prevalent use, contain an oily base, a condition which in itself is undesirable, since it is common knowledge that in many cases some of the oil reaches the lung tissues and there causes decreased effectiveness of the ciliary beat, interstitial fibrosis, giant cell formation and, in extreme conditions, lipoid pneumonia (1, 2).

IMPROVEMENTS IN THE PREPARATION OF INTRANASAL SOLUTIONS

Some attempt has been made in recent years, both by commercial concerns and private workers, to improve and develop more efficacious and plausible nasal preparations. The first step taken has been the substitution of an aqueous base for the oily type. The next and, in most cases, final improvement considered by both the manufacturers and research workers was directed largely at the elimination of the irritant properties of these preparations. With this object in view, the solution was made isotonic with the blood stream, an adjustment which actually only partially removes the irritating effects produced by the common aqueous nasal preparations, since these are, in part, due to the fact that the reaction of the solution does not approximate that of the nasal secretions close enough to be within the range of comfort.

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Recently Robinson and Goldner (3) prepared two isotonic solutions of ephedrine sulfate which they suggested for inclusion in the next edition of the National Formulary. These solutions have several obvious faults: first, the active ingredients are dissolved in physiological solution of sodium chloride in one case and isotonic solution of dextrose and sodium chloride in the other. This method produces solutions which are slightly hypertonic and does not introduce a standard procedure for the manufacture of isotonic solutions containing additional active ingredients without further disturbing the tonicity of the solution. Second, these solutions are not buffered, a condition which, together with the adjustment of the tonicity, entirely eliminates the irritant properties of the common nasal preparations when adjusted to the proper p_H level and which aids materially in the treatment of colds as will be discussed further in the following paragraphs. Another disadvantage of the aforementioned solutions is that they contain but 0.1 per cent ephedrine sulfate which is hardly enough to produce effective constriction of the nasal mucosa. In general practice 1 per cent of ephedrine sulfate is used, and rarely less than 0.5 per cent is employed.

REQUIREMENTS FOR AN IDEAL NASAL PREPARATION

The authors, in an attempt to manufacture a scientific and effective nasal preparation, first set up the specifications to which the ideal preparation should conform and then prepared three solutions containing one-half per cent, one per cent and two per cent ephedrine sulfate, respectively, which embraced the conditions set forth for the ideal solution. A preparation fulfilling these requirements should have the following properties:

1. The solution should contain an effective vasoconstrictor in aqueous solution.

2. The tonicity of the solution should approximate that of the blood stream very closely, thus producing more effective absorption of the active ingredients, as well as partially eliminating its irritant properties.

3. The preparation should be buffered to the p_H level that is consistent with comfort and effective treatment of inflammation of the nasal mucosa.

4. The solution should be adequately preserved in order to prevent the growth of various microörganisms in the preparation which might in some cases cause the subsequent reinfection of the patient.

PREPARATION

Ephedrine sulfate was chosen as the vasoconstrictor because it is so well adapted for use in nasal preparations. This compound has marked vasoconstrictive properties, is stable in aqueous solution and is readily attainable.

The preservative used to prevent the growth of microörganisms in the solution upon exposure to infection was 0.04 per cent of a combination of 65 parts methyl *p*-hydroxy benzoate and 35 parts of propyl *p*-hydroxy benzoate. This compound produces no irritation and has proved to be effective (4). Butyl *p*-hydroxy benzoate (Butoben, Merck) in 0.01 per cent concentration can also be used as the preservative; however, this compound produces slight irritation in particularly sensitive individuals.

To obtain an isotonic solution, buffered at the proper p_H level, two separate isotonic 0.5 per cent ephedrine sulfate buffer solutions were prepared. One solution consists of an isotonic alkaline buffer solution, buffered with dibasic sodium phosphate and the other consists of an isotonic acid buffer solution, buffered with monobasic potassium phosphate. The phosphate salts were chosen as buffer system because they are one of the natural buffer systems of the blood and also because of their great buffering power.

These two buffered isotonic solutions were then titrated one against the other until the proper p_H was attained as measured with an accurate Beckman p_H meter. The p_H of the final product was adjusted to 6.7; this p_H level was obtained by mixing equal parts of the two buffer solutions.

The ideal isotonic nasal preparation, in so far as the removal of the irritant properties is concerned, is one whose p_H is the same as that of the nasal secretions (approximately 7.2). The above solution was adjusted to a p_H of 6.7 because experimental results proved that a solution which is within the p_H range of 6.0 to 7.6 is also non-irritating. In addition, a solution which is slightly acid in reaction according to work recently performed by Fabricant (5) is much more effective in the treatment of colds and sinus troubles. This can be explained by the fact that the nasal and sinus secretions of patients afflicted with colds tend to become more strongly alkaline than normal, and hence the most effective manner in which to relieve the inflammation and congestion is to acidify the nasal passages.

The tonicity of the solution was adjusted using the method suggested by Nicola (6) which sets forth a formula for the calculation of the amounts of salts to be added to a given solution to make it isotonic with the blood stream. The general formula employed is as follows:

$$\frac{\text{Mol. wt. of salt to be used to make solution isotonic}}{\text{Dissociation constant of solute to be used to make solution isotonic}} = \text{Number of Gm. of solute to add per 100 ml. of solution to make it isotonic}$$

$$\text{Number of Gm. of solute to add per 100 ml. of solution to make it isotonic} = \left(\text{0.027026 (Isotonic factor of blood stream)} - \frac{\text{Percentage of active ingredient w/v in solution}}{\text{Mol. wt. of active ingredient in solution}} \times \text{Approximate dissociation constant of active ingredient}\right)$$

ISOTONIC ACID BUFFER SOLUTION FOR 0.5 PER CENT EPHEDRINE SULFATE

$\frac{0.5}{428.33} \times 1.0 = 0.001167$ (tonicity factor for ephedrine sulfate)

$\frac{1.0}{136.14} \times 1.5 = 0.01102$ (tonicity factor for $K-H_2PO_4$)

$\frac{0.3}{74.55} \times 1.5 = 0.006$ (tonicity factor for K-Cl)

$0.027026 - (0.001167 + 0.01102 + 0.006) = 0.008839$

$\frac{0.008839 \times 180.09}{1.0} = 1.5918$ (Gm. dextrose to each 100 cc. of solution)

The average p_H of this solution was found to be 4.42.

ISOTONIC ALKALINE BUFFER SOLUTION FOR 0.5 PER CENT EPHEDRINE SULFATE

$\frac{0.5}{428.33} \times 1.0 = 0.001167$ (tonicity factor for ephedrine sulfate)

$\frac{1.0}{142.02} \times 2.0 = 0.01408$ (tonicity factor for Na_2HPO_4)

$\frac{0.3}{58.45} \times 1.86 = 0.009547$ (tonicity factor for NaCl)

$0.027026 - (0.001167 + 0.01408 + 0.009547) = 0.002232$

$\frac{0.002232 \times 180.09}{1.0} = 0.4020$ (Gm. dextrose to each 100 cc. of solution)

The average p_H of this solution was found to be 8.25.

From these calculations and the fact that the two buffer solutions were mixed in equal parts to attain the desired p_H, 6.7, the following formula for preserved, isotonic, buffered ephedrine nose drops was obtained and is suggested for inclusion in the next edition of the National Formulary.

0.5 PER CENT EPHEDRINE SULFATE

Ephedrine sulfate	0.5 Gm.
Potassium phosphate monobasic	0.5 Gm.
Sodium phosphate dibasic	0.5 Gm.
Potassium chloride	0.15 Gm.
Sodium chloride	0.15 Gm.
Dextrose, anhydrous	0.9969 Gm.
Preserved water, a sufficient quantity, to make	100.00 cc.

If a one per cent solution of ephedrine sulfate is desired, the amount of dextrose in the above formula is reduced to 0.7867 Gm., and for a two per cent solution the dextrose is reduced to 0.3663 Gm.

The quantity for each component of the above formula must be weighed accurately on an analytical balance and made up to the indicated volume in a volumetric flask in order to obtain the desired tonicity and p_H values. In addition the chemicals used must be of the highest purity. The chemicals employed by the authors were all Merck's Reagent brand.

SUMMARY

1. Several improvements in the manufacture of nasal solutions have been developed. These include the substitution of water for the oily base and the adjustment of the osmotic pressure to approximate that of the blood.

2. Specifications for an ideal vasoconstrictive nasal preparation are advanced.

3. The method of manufacture, the calculations involved and the formula for such an ideal nasal preparation are submitted.

4. It is recommended that the formulas submitted for one-half, one and two per cent ephedrine sulfate intranasal solutions be included in the next edition of the National Formulary.

BIBLIOGRAPHY

(1) Proetz, A. W., *Ann. Otology, Rhinology and Laryngology*, 43 (1934), 457.

(2) Laughlen, G. F., *Am. J. Path.*, 1 (1925), 407.

(3) Robinson, Sister Gladys, and Goldner, K. J., JOUR. A. PH. A., *Pract. Pharm. Ed.*, 1 (1940), 410.

(4) Hasler, W. T., *Am. J. Ophthalmol.*, 22 (1939), 423.

(5) Fabricant, N. D., *American Druggist*, 102 (1940), 58.

(6) Nicola, F., *Giron. farm. chim.*, 70 (1921), 57.

"Read not to contradict and confute; nor to believe and take for granted; nor to find talk and discourse; but to weigh and consider".—Sir Francis Bacon

The Fungistatic Value of Certain Ointments*,†

By Francis J. O'Brien and William J. Bonisteel‡

Numerous bacteriological investigations have been made for the purpose of determining the fungicidal value of various substances in solution form. As a result of some of these investigations (1) the conclusion has been reached that there is a close relationship between the bactericidal and the fungicidal action of most substances but that there are exceptions to this rule. Copper salts, for example, are good bactericides but poor fungicides (2, 3). Thymol, oil of cinnamon and oil of clove are much more efficient as fungicides than as bactericides (4).

Other investigations have shown that a substance having a high bactericidal value in solution form may have little or no value when used in the form of an ointment or that its value may depend upon the base into which it has been incorporated (5, 6). Phenol, for example, although highly bactericidal in solution form, loses this value completely when incorporated into a base of petrolatum, but retains it when incorporated into a vanishing cream base.

The primary purpose of this investigation was to determine the fungistatic value of some of the official ointments and certain other non-official ointments prepared from substances which have known fungicidal value in solution form. The secondary purpose was to determine whether the base used in the preparation of the ointment had any effect on the fungistatic value.

The official ointments of iodine, ammoniated mercury, yellow mercuric oxide, phenol, sulfur and compound benzoic acid, and non-official ointments made from thymol, oil of cinnamon and chlorthymol, were tested for fungistatic value.

EXPERIMENTAL

Method Used.—Since there is no standard in the literature for testing the fungistatic property of

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† An abstract of a thesis submitted to the Biology Department of Fordham University by Francis J. O'Brien in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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ointments, the F. D. A. agar plate method was tried using two organisms frequently found in fungous infections, *Monilia albicans* and *Trichophyton interdigitale*. Both of these organisms were obtained from the American Type Culture Collection of Georgetown University, Washington, D. C. They were designated as *Endomycopsis albicans* A. T. C. C. 2091 and *Epidermophyton interdigitale* A. T. C. C. 4808. In the beginning, *Microsporum audouini*, strain M 31, was also used but was found to be the most sensitive of the three organisms to the action of the ointments and the tests with this organism were therefore discontinued.

Although the directions of the F. D. A. method were followed as closely as possible, the results varied so widely that certain modifications were deemed necessary. After a number of tests with *Monilia albicans* as the test organism, the following modifications were found necessary.

1. A definite quantity of ointment should be used in the test when comparative values are desired, since the width of the clear zone usually increased as the amount of ointment used was increased.

2. The ointment should be spread over a definite area of media. It was found that the same amount of ointment gave a greater zone of inhibition when the area of media covered was increased.

3. The ointment should be spread evenly. Uneven spreading of the ointment caused variations in the width of the clear zone, the greater width usually being in that part nearest to the greater thickness of ointment.

4. Since fungi grow more slowly than bacteria accurate readings could be made only after a period of about two weeks' incubation.

5. Incubation carried on at room temperature should not be allowed to vary more than 2° C. during the first 48 hours.

With these factors in mind the following method was devised and used with satisfactory results in all of the tests:

The spores and hyphæ of a 20-day-old culture of the organism were washed off with 10 cc. of sterile saline solution. The mixture was transferred to a test-tube and thoroughly shaken for five minutes with a few glass beads to break up any clumps. It was then strained through three layers of gauze and 5 cc. of the strained mixture added to 400 cc. of Sabouraud's maltose agar previously melted and cooled to about 45° C. Fifteen cc. of the inoculated agar were poured into a sterile petri dish and allowed to harden. Exactly 0.5 Gm. of the ointment to be tested was spread as evenly as possible with a glass rod over the surface of a circular cover glass having a diameter of 18 mm. After applying the ointment on the cover glass to the media, the cover glass was pressed down gently with a glass rod in order to spread the ointment as evenly as possible under but not beyond the cover glass. (Stiff ointments were warmed slightly before applying to secure better surface contact.) An incubation period of 14 days at room temperature was allowed. The result of the test was based on the width of the clear zone surrounding the ointment at the end of this period.

Determination of the Fungistatic Effect of Ointments.—Using the method previously described, five separate tests were made on each ointment during a period of three months. *Monilia albicans* and *Trichophyton interdigitale* were used as the test organisms. All ointments were freshly prepared at the beginning of the series of tests.

Table I shows the average results of the five tests made on each ointment with each organism.

Table I.—Fungistatic Effect of Certain Ointments

Ointment Used	Organism Used: *Monilia albicans*	Organism Used: *Trichophyton interdigitale*
	Clear Zone in Mm.	
Benzoic acid compound	7	10
Yellow mercuric oxide	0	0
Yellow mercuric oxide (double strength)	0	1
Iodine	1	5
Ammoniated Mercury	0	2
Sulfur	0	1
Phenol	0	0
Oil of cinnamon 2%*	11	No growth
Thymol 5%*	12	No growth
Thymol 5% and oil of cinnamon 2%*	12	No growth

* The base of these ointments was composed of petrolatum 90 parts, wool fat 5 parts and yellow wax 5 parts.

Discussion of the Results.—The ointments containing either 5 per cent of thymol or 2 per cent of oil of cinnamon were found to have a higher fungistatic value than any of the other ointments tested. Of the official ointments, benzoic acid compound was found to be the most effective. Ointments of yellow mercuric oxide and ammoniated mercury had no fungistatic value against *Monilia albicans* and had but slight value against *Trichophyton interdigitale*. Iodine was fairly effective against the latter organism but had little effectiveness against the former. It should be noted here that Reddish and Wales (7) and later Husa and Radin (8) found all three of these ointments to be highly bacteriostatic.

Phenol, although highly fungicidal in solution form, was found to have no inhibitory power in the form of the official ointment against either organism. Prout and Strickland (9) have reported that phenol ointment is without bacteriostatic value.

Figures 1, 2 and 3 show the photographic results of some of the tests.

Effect of Ointment Base on the Fungistatic Value of Certain Ointments Against Monilia Albicans.—In order to determine whether the base used had any effect on their fungistatic value, ointments of oil of cinnamon, thymol, chlorthymol and phenol were made with cold cream, vanishing cream, and petrolatum, wax and lanolin bases, and each ointment was tested against *Monilia albicans*. Thus each

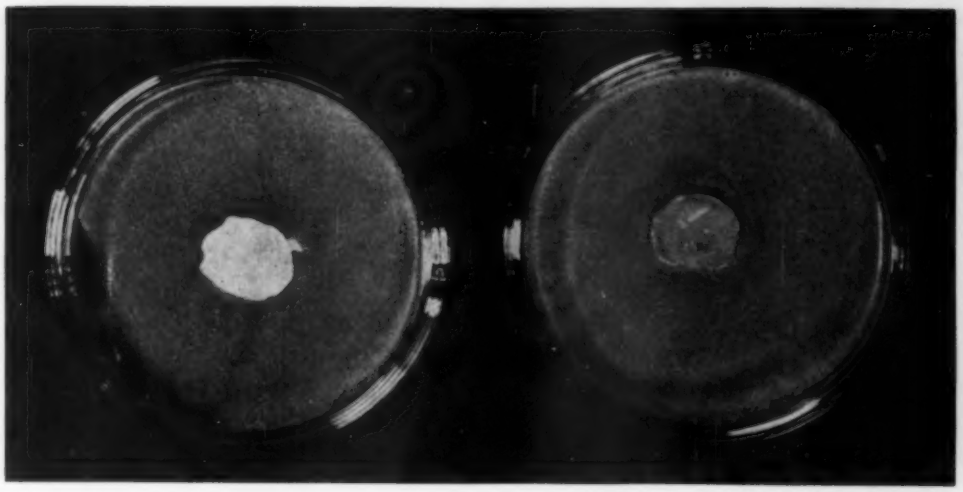

Fig. 1.—Ointment of Ammoniated Mercury (Left) and Ointment of Yellow Mercuric Oxide (Right) Showing no Fungistatic Property against *Monilia albicans*.

ointment was tested in three different bases which were prepared in the usual manner from the following formulas.

COLD CREAM

White wax	25.0 Gm.
Ceresin	12.5 Gm.
Mineral oil	120.0 cc.
Borax	1.2 Gm.
Water	50.0 cc.

VANISHING CREAM

Stearic acid	50.0 Gm.
Triethanolamine	2.0 cc.
Wool fat	5.0 Gm.
Glycerin	15.0 cc.
Water	130.0 cc.

PETROLATUM BASE

Yellow wax	5.0 Gm.
Wool fat	5.0 Gm.
Petrolatum	90.0 Gm.

In the preliminary tests, *Trichophyton interdigitale* was also used as a test organism. It was, however, so sensitive to thymol, oil of cinnamon and dichlorothymol that no growth appeared on any of the plates and comparison was impossible.

A test was made to determine whether the growth of *Trichophyton interdigitale* could be inhibited by the vapors only of the ointments of thymol, oil of cinnamon or chlorthymol. Maltose agar slants were inoculated with the organism and 1 Gm. of the ointment to be tested was placed on the tip of the cotton plug. The plug was then inserted into the

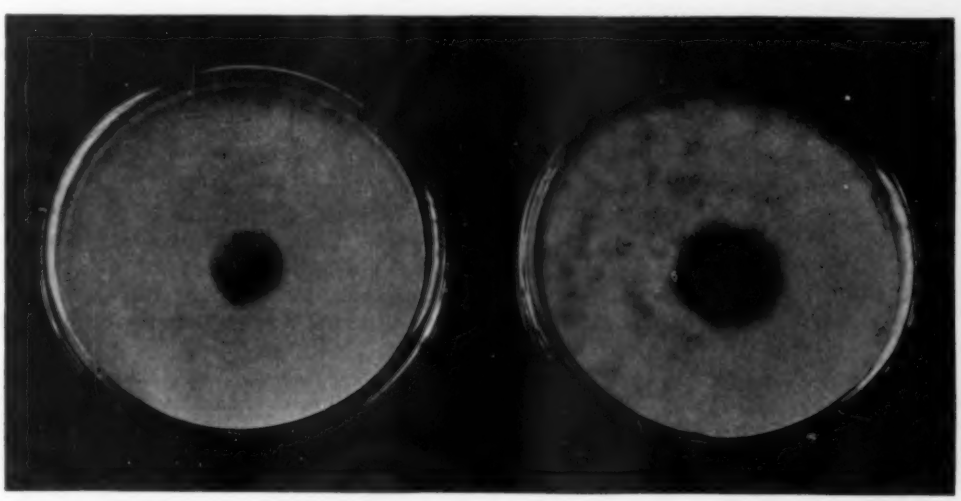

Fig. 2.—Ointment of Phenol (Left) Showing No Fungistatic Property against *Trichophyton interdigitale* and Ointment of Yellow Mercuric Oxide, 2 Per Cent (Right) Showing but Slight Value against the Same Organism.

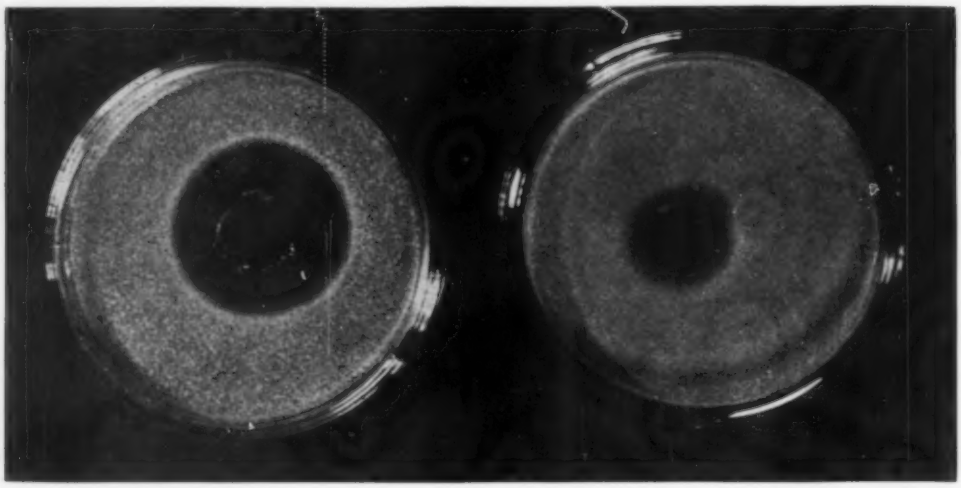

Fig. 3.—Iodine Ointment with 5 Per Cent of Thymol and 2 Per Cent of Oil of Cinnamon Added (Left) Showing Good Fungistatic Property against *Monilia albicans* and Plain Iodine Ointment (Right) Showing Only Slight Fungistatic Value against the Same Organism.

test-tube so that the ointment remained at least one inch away from the media. The tube was firmly corked, the cotton plug and the ointment being left in the tube. No growth was observed after 14 days of incubation at room temperature when ointments containing one per cent of oil of cinnamon or two and one-half per cent of thymol were used. Ointments made with two per cent of phenol or with two per cent of chlorthymol did not prevent growth.

In a similar test, *Monilia albicans* was not inhibited by any of the ointments.

Trichophyton interdigitale was therefore discontinued as a test organism and *Monilia albicans* which was much more resistant was used.

Five tests were made at different times with each ointment. In each test the ointments were identical except for the base used. Table II shows the variation in the width of the clear zone when the same ointment in a different base was tested against *Monilia albicans*.

Table II.—Fungistatic Value of Substances in Different Bases When Tested against *Monilia Albicans*

Substances Tested	Base Used		
	Cold Cream	Vanishing Cream	Petrolatum, Wax, and Wool Fat
	Clear Zone in Mm. (Average of 5 Tests)		
Cinnamon oil 2% Thymol 5%	16	20	12
Cinnamon oil 1% Thymol 2½%	14	16	9
Thymol 5%	15	17	12
Thymol 2½%	14	16	5
Cinnamon oil 2%	16	18	11
Cinnamon oil 1%	14	17	9
Chlorthymol 2%	4	10	3
Phenol 2%	4	5	0

Discussion of the Results.—In all of the ointments tested, the vanishing cream base permitted the greatest diffusion of the antiseptic as measured by the width of the clear zone around the ointment. Cold cream bases were only slightly inferior in this respect except in the case of chlorthymol ointment. The least diffusion was permitted by the base composed of petrolatum, wool fat and wax.

Ointments of oil of cinnamon or thymol were distinctly superior in fungistatic value to either ointments of chlorthymol or phenol. Phenol was without value in the petrolatum base, but was slightly effective in a base of either cold cream or vanishing cream.

The ointments containing 2½ per cent of thymol were just about as effective as those containing 5 per cent except in the petrolatum base. One per cent of oil of cinnamon was only slightly less effective than 2 per cent in any of the bases used.

It was observed that the growth of the organism outside of the clear zone was always less dense when ointments containing thymol were used than when any of the other ointments were used. This suggests the possibility that thymol may diffuse over a larger area than recorded by the clear zone in sufficient concentration to be slightly inhibitory.

None of the bases used had any inhibitory power in themselves, although cold cream did delay the growth of the organism for a period of about two days in a zone of 5-mm. width. It must therefore be concluded that vanishing cream and cold cream bases permitted a greater diffusion of the antiseptic into the media than did the petrolatum base.

Fungistatic Value of Some Commercial Ointments.—In order to check the relative efficiency of thymol and oil of cinnamon ointments with some of the commercial ointments used in treatment of fungus infections, a series of tests similar to those carried out previously was made. Table III shows the results of these tests in terms of the clear zone surrounding each ointment.

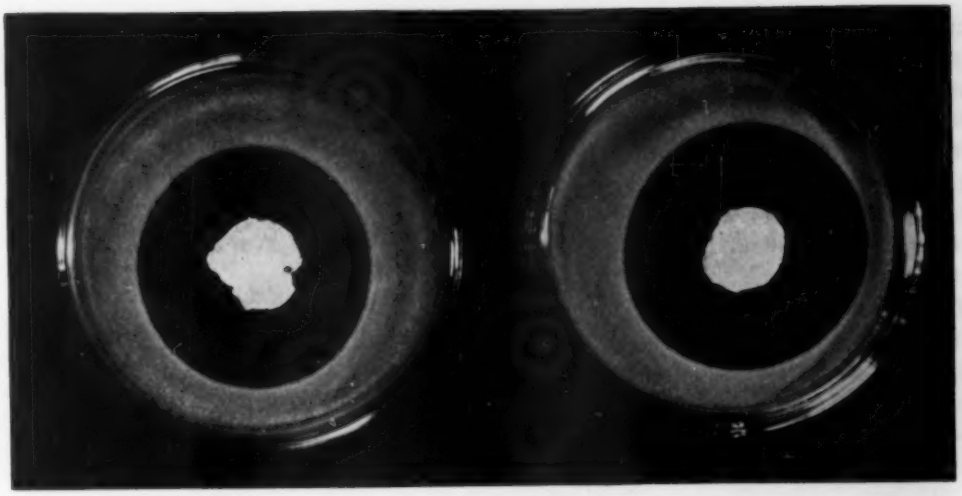

Fig. 4.—Showing Good Zones of Inhibition of *Monilia albicans* with a 2 Per Cent Ointment of Oil of Cinnamon in a Cold Cream Base (Left) and in Vanishing Cream (Right).

Table III.—Fungistatic Effect of Three Commercial Ointments

Ointment Used	Clear Zone in Mm. (Average of 5 Tests)
Merthiolate (1–2000)	11
Kerolysin	7
Fungi rex	7

Comparison with the preceding table shows that either a $2^1/_2$ per cent thymol ointment or a 1 per cent oil of cinnamon ointment in either cold cream or vanishing cream bases is superior in fungistatic value to any of the commercial products tested when *Monilia albicans* is used as the test organism.

CONCLUSIONS

It is to be concluded that (1) a bacteriostatic ointment may or may not be fungistatic, (2) the fungicidal value of a substance in solution form is not an indication that it will have the same value or any value at all in ointment form, (3) the fungistatic effect of a substance in ointment form may depend upon the base into which it is incorporated.

In addition to the above general conclusions, it is also concluded that (1) the U. S. P. ointments of phenol, yellow mercuric oxide, ammoniated mercury, sulfur and iodine have little or no fungistatic effect when tested against *Monilia albicans* or *Trichophyton interdigitale*, (2) ointment of benzoic acid compound N. F. has decided

Fig. 5.—Showing the Wider Zone of Inhibition of *Monilia albicans* Produced by a $2^1/_2$ Per Cent Thymol Ointment in a Base of Cold Cream (Left) than by a 2 Per Cent Chlorthymol Ointment in the Same Base (Right).

fungistatic property, (3) thymol and oil of cinnamon show excellent fungistatic action in any of the bases used but particularly in vanishing cream or cold cream, (4) ointments of thymol or oil of cinnamon or a combination of both in either cold cream or vanishing cream are superior to any of the ointments tested including three commercial ointments.

The favorable results of the *in vitro* reactions of thymol and oil of cinnamon seem to indicate that a clinical trial is warranted. The following specific formula and certain modifications of it are suggested.

Thymol	2.5 Gm.
Oil of cinnamon	1.0 cc.
Cold cream	
or	
Vanishing cream *q. s.*	100.0 Gm.

Cold cream should be used as the base when the soothing effect of an oily substance is desired or when the addition of salicylic acid is desired.

U. S. P. iodine ointment may be used as the base if the bacteristatic action of iodine is desired in addition to the fungistatic action of the thymol and oil of cinnamon.

REFERENCES

(1) Woodward, G. J., *et al.*, *J. Lab. Clin. Med.* 19 (1934) 1216.

(2) Emmons, C. W., *Arch. Dermatol. and Syphilol*, 28 (1933) 15.

(3 DeWitt, L. M., and Sherman, H. J., *J. Infect. Diseases*, 18 (1916) 369.

(4) Meyers, H. B., *J. Am. Med. Assoc.*, 89 (1927) 1934.

(5) Gershenfeld, L. and Miller, R. E., *Am. J. Pharm.*, 105 (1933) 186.

(6) *Ibid.*, 105 (1933) 194.

(7) Reddish, G. F., and Wales, H., JOUR. A. PH. A., 18 (1929) 576.

(8) Husa, W. J., and Radin, J. M., *Ibid.*, 21 (1932) 861.

(9) Prout, W. A., and Strickland, M., *Ibid.*, 26 (1937) 730.

"To know things well, one must know them in detail, and as this is infinite, our knowledge is necessarily superficial."—La Rochefoucauld.

Ointments Prepared by Emulsification

Improvements and Advantages Gained; Choice of Emulsion Systems; Selection of Vehicles

*By A. J. Gibson, Ph.C., H. E. Parker, B.S.Pharm., and Anne Almus, A.B.**

For the purpose of this paper there are two basic methods of preparation of ointments regardless of the vehicles chosen in formulating the finished product. (1) They are those ointments made without water (non-emulsified) and (2) those made with water (emulsified). As will be shown, the second seems to offer the most practical approach to improvement in ointment compounding whether it be from the medical or pharmaceutical viewpoint. Recognition of the advantages of the emulsified type of ointment are becoming apparent in medical circles. Recently Fantus (1) pointed out that ointments are contraindicated in acute inflammatory conditions of the skin because of their "heating quality." He elaborates further and shows that the exception to this is in the use of cream ointments or emulsions. In fact, this clinician states that the success or failure of the treatment used may depend on the proper choice of the ointment base. Mumford (2), in discussing the role of emulsifying bases in dermatology, observes that the application of ordinary bases as petrolatum, lanolin or mixtures of these offers an objectionable barrier for the serous discharge of the skin. Contrasting this with the advantages of emulsions in overcoming this objection, he shows the superiority of emulsified ointments as carriers for both oil-soluble and water-soluble ingredients. Emphasis is placed on the lack of progress in prescribing ointments; in fact, Mumford goes so far as to state "progress in prescribing ointments has not proceeded far beyond the lanolin and vaseline of fifty years ago." Traub (3) calls attention to one of the prime reasons why patients do not follow instructions and quickly tire of the ointments prescribed—because

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the active ingredients are incorporated in simple greasy bases unsuited for convenient application and removal.

Bamber (4) is in agreement with Fantus and Mumford in stating that ointments applied to the skin must not interfere with its normal function of radiation of heat and secretion. His own observations show that antiseptics usually give better results in emulsions than when combined in simply greasy bases. It becomes clear, therefore, that interest in improvement of ointment bases is by no means confined to the pharmaceutical profession. It is becoming increasingly evident that medical circles are demanding it, partly due, no doubt, to pres-

Fig. 1.—Test Organism: *Microsporon lanosum*. Period of Incubation: Seven Days at 20° C.

Sabourraud medium
Plate method
Width of zone: none

Fig. 2.—Test Organism: *Microsporon lanosum*. Period of Incubation: Seven Days at 20° C.

Sabourraud medium
Plate method
Width of zone: 19 mm.

Fig. 3.—Test Organism: *Trichophyton gypseum*. Period of Incubation: Seven Days at 20° C.

Sabourraud medium
Plate method
Width of zone: 14 mm.

Fig. 4.—Test Organism: *Trichophyton gypseum*. Period of Incubation: Seven Days at 20° C.

Sabourraud medium
Plate method
Width of zone: 25 mm.

sure from the public in logically expecting their local medication in a less obnoxious form. This fact is borne out by the experience of the authors in this laboratory in filling a large number of dermatological prescriptions over a ten-year period. It has been the experience of our staff physicians and the specialists in dermatology that better coöperation is obtained from the patients if the ointments are prepared in an emulsified manner. It has been found that they do not stain as readily, are less conspicuous when applied, and are easier to remove in washing from the skin or where the clothing comes in contact with the material.

Aside from the point that emulsified ointments do not seem to interfere in the same degree with the physiological processes of the skin secretions as previously indicated, and they are cosmetically more acceptable to the patient, there is another factor that can be illustrated to advantage. Better results are secured with the bacteriostatic or bacteriocidal agents when incorporated in this newer manner as measured with the agar plate or agar cup method. Kuever and Burnside (5) prepared a W/O ointment emulsion of mercuric nitrate of the same percentage as the N. F. VI. They found that their preparation was three times more effective as measured by the agar cup method, using *Staphylococcus aureus* as the test organism. Gershenfeld and Brillhart (6) recently studied some of the synthetic wax and oxycholesterin bases as emulsifiers for ammoniated mercury, phenol and mercuric chloride. They conclude that these bacteriocidal agents produce better results when emulsified in the newer bases as compared to the same agents in benzoinated lard, petrolatum, lanolin and the U. S. P. XI base. In evaluating the results, the F. D. A. agar plate and cup method with *Staphylococcus aureus* was used. Additional work here in this laboratory extending back some five years, bears out the investigation of Kuever and Burnside, Gershenfeld and Brillhart.

EXPERIMENTAL

In an attempt to appraise the effect of emulsified ointments and non-emulsified ointments on other than *Staphylococcus aureus*, the authors selected two mold cultures, *Microsporon lanosum* and *Trichophyton gypseum*. These were obtained from clinical cases from the department of dermatology of the University of Michigan Hospital. Our reasons for selecting these mold cultures were twofold. First, *Microsporon lanosum* is very commonly present in seborrhoea of the scalp. *Trichophyton gypseum* is one of the fungi responsible for tinea of the feet and hands. Secondly, both are very resistant to ordinary chemical agents commonly used in the treatment of the lesions caused by these fungi in man. Both groups of formulas listed below have been used in treating these infections, by the physicians on the staff for five years or longer. Those treated were student patients presenting themselves at the Health Service with the typical clinical picture of seborrhoea of the scalp or tinea of the feet or hands. It is hardly within our province to discuss any clinical results obtained with these emulsified ointments as contrasted with the same formulas of such commonly known ingredients incorporated in the U. S. P. XI base. However, we can demonstrate the distinctly superior results of the growth-inhibiting properties of the emulsion ointments on the two cultures named and illustrated in the photographs herein.

Formulas

No. 1		Per Cent
	Sulfur Praec.	5.0
	Acid Salicyl.	2.0
	Base U. S. P. XI	93.0
		100.0

No. 2		Per Cent
	°Tegacid[1]	8.0
	Cetac.	7.0
	Ol. Theobrom.	5.0
	Petrolat. Liq.	8.0
	Acid Salicyl.	2.0
	Sulfur Praec.	5.0
	Aq. Dest.	65.0
		100.0

No. 3		Per Cent
	Acid Salicyl.	3.0
	Acid Benz.	6.0
	Base U. S. P. XI	91.0
		100.0

No. 4		Per Cent
	°Tegacid	15.0
	Ol. Theobrom.	10.0
	Petrolat. Liq.	3.0
	Acid Salicyl.	3.0
	Acid Benz.	6.0
	Aq. Dest.	63.0
		100.0

Observing sterile technique, two poured plates were made with Sabourraud's medium and each of the molds (*Microsporon lanosum* and *Trichophyton*

[1] A type of Glyceryl Monstearate supplied by Goldschmidt Corp., New York, N. Y.

gypseum). On hardening, 1-Gm. samples of ointment No. 1 in U. S. P. base and No. 2 emulsified base were placed on the Microsporon plates. One-gram samples of ointment No. 3 in U. S. P. base and No. 4 emulsified were placed on the Trichophyton cultures. The plates were incubated at 20° C. for seven days and then photographed.

DISCUSSION

To recapitulate then the advantages of emulsified ointments compared to those not emulsified: (1) It has been shown that they do not interfere in the same degree with the normal function of the skin secretions. (2) There is reason to believe that they are more effective when applied. (3) They are certainly more acceptable cosmetically from the patient's viewpoint. (4) They are more effective as bacteriostatic or bacteriocidal agents when tested by the agar plate, agar cup or other acceptable procedures. If, then, ointment emulsions have decided advantages, what is the most desirable emulsion system to use and what type of emulsifiers should be selected? According to Clayton (7), who quotes Selmi and Hatschek, an emulsion is a system containing two liquid phases one of which is dispersed as globules in the other. Two types of systems are possible, water in oil or oil in water (8). The official cold cream is an example of the W/O type. Excellent as a cosmetic cream it is not suitable, or for that matter intended, as a base for ointments wherein the prescriber may wish to use a variety of therapeutic agents as benzoic and salicylic acids in Whitfield's ointment. It is obvious that the sodium soaps resulting from the reaction of sodium borate and the fatty acids of the waxes in the U. S. P. cold cream are incompatible with many of the every-day medicaments.

At this point attention is called to the premise that the type of emulsifier used will determine the type of emulsion formed (9). Therefore, for a W/O emulsion a hydrophobic colloid is indicated (10). In an emulsion of O/W the converse is true, namely, the hydrophilic colloid is the one of logical choice. Adherence to this cardinal principle will save time and trouble for the investigator in avoiding pitfalls in attempting to formulate emulsions using antagonistic emulsifiers (11). Powers, Leask and Warner (12) in a recent paper have added valuable information on hydrophobic colloids as emulsifiers for emulsions of W/O. They demonstrated that mixtures of cholesterols and cholesterol esters increase markedly the emulsion-forming properties of cholesterol. Fiero (13) calls attention to the use of hydrogenated oils in emulsions for formulating ointment bases. Much interest has been shown in late years by the pharmaceutical and medical professions in oxycholesterin so-called "absorption bases." Johnston and Lee (14) investigated these bases as vehicles in W/O emulsions for ointment use. They conclude that aside from extemporaneous use, they are sensitive to temperature changes and crack readily. The experience of the writers confirms this, in that absorption bases, as such, are not practical for ointment emulsions. We have found that attractive-appearing cosmetic creams can be formulated with twenty to thirty per cent of the material with as high as sixty-five per cent water. Even this emulsion, devoid of medicaments, did not shelf test well. If water contents of sixty to sixty-five per cent were used, the cream developed a granular appearance with slight "sweating" of water on long standing. The spreading quality was poor. Drag was noticeable. If the water content of the cream was reduced to a level of forty to fifty per cent, the emulsion developed "bleeding" of the oil fraction. Our experience covered the use of such proprietary bases as Protegin, Protegin X and Aquaphor.

Apparently the most logical approach to formulating stable ointment emulsions is with the O/W type. They are easy to formulate and from a cosmetic standpoint they approach the ideal in consistency, color and spreading qualities. Inherently, this emulsion system possesses a stability that has much to recommend it. It is possible to incorporate large amounts of water without upsetting the equilibrium of the two phases; a total of seventy per cent as the dispersing phase is not at all unusual. The importance of the water content has been set forth by Prout, Eddleman and Harris (15) in a study of silica gel as a vehicle for different germicides. They believe that high percentages of water allow greater diffusion of the antiseptic from the ointment base and enhance its efficiency when applied. From a medical viewpoint, Wise and Wolf (16) in discussing the parasiticidal action of sulfur on the skin conclude that this action is in all probability due to the formation of H_2S. It seems natural that this would be favorably influenced by a high water content in an ointment emulsion. Wood (17) confirms the greater efficiency of O/W emulsions. He studied emulsions of acriflavine in W/O and O/W using a resistant strain of *Staphylococcus aureus* to determine the value of the emulsion. His results show that "emulsions of the O/W type have far greater bacteriostatic and germicidal properties than those of the W/O."

As in the case of W/O alkaline emulsions it would likewise appear advisable to avoid alkaline O/W emulsifiers. As examples, soaps resulting from the reaction of sodium, potassium or ammonium hydroxides with fatty acids are a basis for satisfactory O/W cosmetic creams. However, the ease with which these emulsions hydrolyze will obviously act as a limting factor for their use in ointment creams. The same seems obvious for emulsions of O/W prepared with the hydroxyethylamines and fatty acids. Though the emulsions formulated may be neutral or nearly so, the ease with which they also hydrolyze is a serious objection. The use of finely divided solids as emulsifying agents has been suggested by Tainter, Kulchar and Stockton (18) as well as by Cox and Goedrich (19). Attention is also being drawn to the use of synthetic waxes, high molecular weight fatty alcohols and mixtures of these. Mumford (20)

Soulsby (21), Gershenfeld and Brillhart (22), Clark (23) and Burnside and Kuever (24) have investigated and suggested formulas. Typical examples of proprietary synthetic waxes are diglycol stearate and oleate derived by the esterification of glycols with different fatty acids. Certain esters of polyhydric alcohols with high molecular weight fatty acids have come into wide use as O/W emulsifiers. Representative of these esters are glyceryl monostearate and glyceryl monostearate stabilized toward acids with acylated alkylene diamines. Though the second, known under the trade name Tegacid (25) is useful as a stabilizing emulsifier of acid ointment creams of O/W, the very nature of all of these esters mentioned are such as to have distinct limitations in use. Their action in the presence of alkalies, acids, salts, metals and strong electrolytes is too often unpredictable. In an effort to overcome these objections, we have tried out a large series of emulsifiers with the following objectives:

1. One that would promote O/W emulsions.
2. One that would promote emulsions stable to nearly all medicaments the pharmacist might be called on to incorporate.
3. The emulsifier must be economical to use. The finished emulsion should compare favorably in price with petrolatum.
4. The emulsifier should permit the use of at least fifty per cent water concentration, otherwise the ointment cream approaches petrolatum in greasy characteristics.
5. The material used must lend itself to both extemporaneous and stock preparation of ointment emulsions.
6. The emulsifier to be readily available and its composition known.

The results of our work indicate that a mixture of one-half per cent sodium lauryl sulfate and eight per cent cetyl alcohol appears to fulfil the conditions named. From a practical standpoint cetyl alcohol in itself is not a strong enough emulsifying agent for general use; it requires the use of a booster or auxiliary agent Sodium lauryl sulfate is not only compatible, but in itself is an outstanding emulsifier. The following serves to illustrate the composition of the emulsion minus any medication.

	Per Cent
Sodium lauryl sulfate	0.5
Cetyl alcohol	8.0
Roba[2]	6.5
Petrolatum Alba	20.0
Water	65.0

Procedure.—In preparing extemporaneous ointment emulsions, it has been a time-saving element to have the base prepared beforehand. This consists of the cetyl alcohol, petrolatum and roba previously melted in a suitable container over a water bath, the sodium lauryl sulfate then added and the whole stirred until cool. As calls are received, the water is determined arbitrarily at a fifty per cent ratio of the weight of the preparation to be made. The percentages of medicaments are then weighed out. The combined weight of water and medication subtracted from the total gives the desired amount of base to use. The base and medication are worked together on an ointment plate, the water previously warmed is added by degrees until a smooth cream results. For extemporaneous work it is obvious that the per cent of base will vary with the per cent of medication called for. Dependent on this factor, the amount of sodium lauryl sulfate and cetyl alcohol will vary slightly from the fixed ratio of one-half and eight per cent. Water content has been retained at a fifty per cent ratio in ointment emulsions in which medication is added. There is no reason other than that this water ratio is a convenient amount to work with and gives satisfactory results The following chemicals have been incorporated in this emulsion system, either separately, or combined in varying percentages:

Salicylic acid 3%/c Benzoic acid 6%.
Juniper tar (Oil of cade) 10%/c Salicylic acid 3%.
Coal tar 5%.
Kaolin 10%/c Sulfur precipitated 10%.
Ammoniated mercury 5% and 10%.
Sulfur precipitated 10%.
Phenol 2%.
Sulfathiazole 1%.
Balsam Peru 10%/c Sulfur precipitated 10%.
Salicylic acid 2%/c Sulfur precipitated 5%.
Calamine 8%.

CONCLUSIONS

1. The objections and shortcomings of the simple greasy vehicles for ointment bases have been reviewed from a medical and pharmaceutical standpoint. The need for improvement seems clear.

2. It has been demonstrated that emulsified ointments offer distinct advantages over the older non-emulsified types, both from a medical and pharmaceutical viewpoint. These points have been set forth.

3. Two emulsion systems have been discussed in relation to ointment bases, water in oil and oil in water.

4. It would seem the O/W type of emulsion system is the better choice of the two. The advantages have been explained.

5. A mixture of sodium lauryl sulfate one-half per cent with cetyl alcohol eight per cent has been suggested as a suitable emulsi-

[2] This may be left out. Experience shows that the emulsions are equally good without it. Our purpose in this laboratory was to incorporate it as an inexpensive substitute for cocoa butter. Supplied by Proctor and Gamble.

fier for stable O/W emulsified ointments.

6. A representative group of common medicaments has been incorporated with good results. The water content in all cases was in the ratio of fifty per cent.

REFERENCES

(1) Fantus, B., *J. Am. Med. Assoc.*, 107 (1936), 861–863.
(2) Mumford, P. B., *Brit. J. Dermat.*, 50 (1938), 540–543.
(3) Traub, E. F., *Urol. Cutaneous Rev.*, 43 (1939), 409–411.
(4) Bamber, G., *Brit. J. Dermat.*, 52 (1940), 21–25.
(5) Kuever, R. A., and Burnside, C. B., JOUR. A. PH. A., 29 (1940), 325–327.
(6) Gershenfeld, L., and Brillhart, R. E., *Am. J. Pharm.*, 111 (1939), 430–442.
(7) Clayton, W., "The Theory of Emulsions and Their Technical Treatment" (1935), page 1.
(8) *Ibid.* (1935), 415.
(9) *Ibid.* (1935), 169–170.
(10) Bancroft, W. D., "Applied Colloid Chemistry" (1921), page 262.
(11) Clayton, W., "The Theory of Emulsions and Their Technical Treatment" (1935), pages 171–172.
(12) Powers, J. L., Leask, H. B., and Warner, R. S., JOUR. A. PH. A., 29 (1940), 14.
(13) Fiero, G. W., *Ibid.*, 29 (1940), 187–192.
(14) Johnston, G. W., and Lee, C. O., *Ibid.*, 29 (1940), 236–239.
(15) Prout, W. A., Eddleman, Mae S., and Harris, R. G., *Ibid.*, 29 (1940) 372–373.
(16) Wise, F., and Wolf., J., *J. Am. Med. Assoc.*, 107 (1936), 1132.
(17) Wood, W. C., *Brit. Pharm. J.*, 142 (1939), 327.
(18) Tainter, M. L., Kulchar, G., and Stockton, A. B., JOUR. A. PH. A., 29 (1940), 306.
(19) Cox, C. L., and Goedrich, P., *Ibid.*, Practical Pharmacy Edition, 1 (1940), 210.
(20) Mumford, P. B., *Brit. J. Dermat.*, 50 (1938), 540–543.
(21) Soulsby, J., *Ibid.*, 52 (1940), 25–35.
(22) Gershenfeld, L., and Brillhart, R. E., *Am. J. Pharm.*, 111 (1939), 430–432.
(23) Clark, W. C., *Ibid.*, 111 (1939), 228–233.
(24) Burnside, C. B., and Kuever, R. A., JOUR. A. PH. A., 29 (1940), 373.
(25) Clayton, W., "The Theory of Emulsions and their Technical Treatment" (1935), page 93.

"To read textbooks is easy, but to do research is to grapple, inch by inch, with the obscure, and battle step by step, with the unknown."—Victor Robinson

Book Reviews

Biological Stains, by H. J. CONN and others. 4th Edition. 308 pages. 5¼ x 8⅛. 1940. Geneva, N. Y.: Biotech Publications. $3.40.

The Commission on Standardization of Biological Stains was organized in 1920 and its function is to certify the quality of stains which are used in the United States by bacteriologists, pathologists, clinicians, etc. At present, this Commission is working in coöperation with pharmacy and much of its work is to be included in the next edition of the National Formulary.

The book contains valuable information concerning many important stains, as follows: characteristics and uses of important stains, dye synonyms, formulas for the preparation of solutions used for staining, color index number, Schultz number, relation of stains to their color index number, special techniques and their authors' names, methods of testing the more important stains, etc. The book also contains a large bibliography for staining techniques.—A. G. D.

Biology in the Making, by EMILY EVELETH SNYDER. xii + 539 pages. 5½ x 8. 1940. New York: McGraw-Hill. $2.80.

This book is written in a style which will attract the scientist as well as the layman; and the more important phases of the biological sciences and the biographies of leading scientists are presented.

In each chapter the latest advances in biology and the scientists responsible for these achievements are given. Various illustrations are included. Some of the subjects discussed are: physiology, nutrition, blood anesthesia, chlorophyll, the cell theory, fossils, the germ theory, evolution and heredity, contagious diseases, serums, antitoxins, etc.—A. G. D.

Chemists Dictionary of Synonyms, 136 pages. 5 x 7¼. 1940. London: THE CHEMISTS AND DRUGGISTS. 5s. plus $.75 for handling charges.

This book is prepared and published by *The Chemist and Druggist* and includes many synonyms which are of aid to the average pharmacist in the practice of his profession.

Although this book is based upon Rouse's Synonyms, it should prove itself of value to the pharmacists in the United States.—A. G. D.

Manual of Clinical Chemistry, by MIRIAM REINER. xv + 296 pages. 4¾ x 7. 1941. New York: Interscience Publishers, Inc. $3.00.

This small laboratory manual which is a useful and a practical laboratory outline of clinical chemistry should be of interest to any pharmacist who is contemplating carrying out this type of work in his pharmacy.

Under the heading of blood analysis, gases and enzymes, inorganic constituents, carbohydrates,

nitrogenous constituents, pigments, lipids, etc., are thoroughly covered. A large section is devoted to urinalysis; and all of the common tests which are used in clinical chemistry are given.

Methods are included for the extraction of preparations of antigens from pollen, feathers and hairs of animals, and house dust; the determination of sulfanilamide in the blood and urine; the determination of arsenic; various determinations for biological analysis for sex hormones, some of the vitamins, cerebrospinal fluid, feces, kidney and liver function tests, gastric analysis, the preparation and sterilization of 50% solution of glucose for intravenous use. References are included.—A. G. D.

The Chemical Action of Ultraviolet Rays, by CARLETON ELLIS and ALFRED A. WELLES. Revised and enlarged edition by Francis F. Heyroth. ix + 961 pages. 6 x 9. 1941. New York: Reinhold Publishing Corporation. $12.00.

The average pharmacist is interested in ultraviolet rays (the radiant spectrum below 4000 Angstrom units) as these rays are of pharmaceutical importance in the irradiation of ergosterol for the production of vitamin D_2, the production and prevention of sunburn and in various types of sterilization.

The first part of the book describes the various methods used for the production of ultraviolet rays—sun, open arcs, etc.—and their uses and sources. The latter portion is devoted to the biological applications of ultraviolet rays, applications of photochemistry to industrial problems, photochemical reactions, etc. Many references are included.—A. G. D.

Clinical Pellagra, by SEALE HARRIS, with SEALE HARRIS, JR. 494 pages. 6 3/4 x 9 3/4. 1941. St. Louis: C. V. Mosby Co. $7.00.

Although it is generally thought that pellagra is a nicotinic acid-deficiency disease, the author presents a number of theories for the cause of this condition as insufficiency of the stomach and liver. The maize-toxin theory, photosensitization of pellagrins and the effect of alcohol in pellagra are discussed.

The prevention, diagnosis and treatment of pellagra are given in detail in this book.—A. G. D.

Le Clergé et la Pharmacie. Essai sur le rôle du Clergé et plus particulirement des Congregations religieuses dans la préparation et la distribution des remèdes avant la Révolution; a review of the book by J. TOURNIER I.

The material covers a field much neglected in the writings of our own country. The review covers the introductory chapters dealing with the role of the pagan priesthood and that of monastic orders; edicts and orders concerning remedies and their purveyors; the role of female religious workers in hospital pharmacies; role of the nuns in convent pharmacies. Some of the famous capuchins will be discussed separately in a later report.—Z. M. C.

Modern Drug Encyclopedia and Therapeutic Guide, by JACOB GUTMAN. 6 1/2 x 9 1/4. xxiv + 1644 pages. 1941. New York: New Modern Drugs. $7.00.

This new edition represents a quick reference desk service on over eleven thousand ethical specialties distributed by nearly three hundred manufacturers. For each specialty the composition, action and uses, the name of the manufacturer, how marketed, the method of administration, etc., are briefly summarized. For quick reference, three indices (Drug, Manufacturers' and Therapeutic) are given. Free supplementary service issued every three months, complete with cumulative index is included.—A. G. D.

Principles and Practice of Chromatography, by L. ZECHMEISTER and L. CHOLNOKY. Translated into English by A. L. BACHARACH and F. A. ROBINSON. 362 pages, 5 x 8. London: Chapman and Hall, Ltd., 11 Henrietta Street, W. C. 2. 25*s*.

This book discusses in detail this new method (principles of the technique and applications) of separating organic chemicals based upon their differences of adsorption affinity. Procedures, types of apparatus, solvents, adsorbents, etc., are given and are thoroughly discussed.—A. G. D.

The Chemical Constitution of Natural Fats, by T. P. HILDITCH. xi + 438 pages. 1940. London: Chapman and Hall Ltd. 35*s* net.

In this book the author has attempted to classify natural oils and fats from the biological as well as constitutional points of view. The natural oils and fats have been grouped under the following headings: (1) Fats and aquatic origin (*a*. Fresh water, and *b*. Marine); (2) Fats of land animals; (3) Milk Fats; and (4) Vegetable fats (*a*. Seed fats, *b*. Fruit pulp fats and *c*. Fruit carnel fats). The characteristic differences in their compositions (fatty acid and glycerides) have been given; and the method of classifying oils and fats into the non-drying, semi-drying and drying groups has been discarded.

This book is based primarily on the process of oxidation of natural fats and oils which has been carried out by Hilditch and his co-workers on the determination of the nature and the respective positions of the component fatty acids. Many representative natural oils and fats are discussed.

Research workers will find this book of much value and it is a very good reference book for workers in the complicated field of oils. (From *Jour. Indian Chem. Soc.*, 17 (1940), 730.)—F. J. S.